THE MEDICINAL CHEMISTRY OF SOME STEROIDAL INDOXYLIDENE CARBOXYLIC ACIDS AND DERIVATIVES

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DECLARATION

'This thesis is my original work and has not been presented for a degree in any other University.'

F. N. KAMAU

'This thesis has been submitted for examination with our approval as University supervisors.'

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DEDICATION

To my family, for their patience and encouragement.

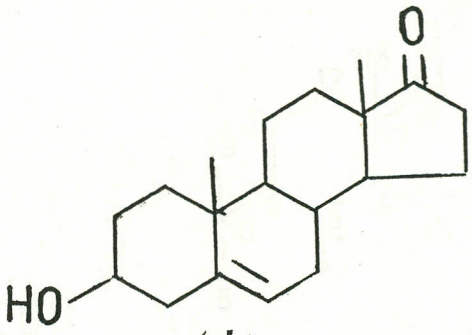
NOMENCLATURE

The nomenclature adopted in the designation of the various compounds in this project is based on that used by Hassner, Haddadin and Catsoulacos (1966). For example (5) which was synthesised from 3β-hydroxy-5-androsten -17-one (4) by Hassner et al (1966) was named 3β-hydroxy-16, 17-seco-16-nor-5-androsten-15-(2' -indoxyliden) -17- oic acid. In an analogous manner, (15), derived from 5α- androstan-17β -ol -3-one (14), and is a novel compound, is named 17β - hydroxy -2,3-seco-2-norandrostan-1-(2' indoxy-liden)-3-oic acid.

The lactam (6), obtained by cyclisation of 5, was named as a derivative of (5) by Hassner et al (1966), namely 3β -acetoxy-16,17-seco-16-nor-5,14-androstadien-15-(3' -acetoxy-2' -indolyl)-17-oic acid lactam. Analogously, The lactam (16), derived from (15), is named 17β-acetoxy-2,3-seco-2-norandrostan-1-(2' -indoxyliden)-3-oic acid lactam.

The indoles derived from (5) and (15), and the lactams synthesised from them, are named in a similar manner, the only difference being the substitution of the word indolyl for indoxyliden.

This nomenclature is not necessarily the I.U.P.A.C. nomenclature, but it allows in the discussion the direct comparison of these compounds with steroids since the numbering of the carbon atoms remains unchanged. This has proved useful in structural elucidation of the synthesised compounds, especially by ^{1}H-NMR,



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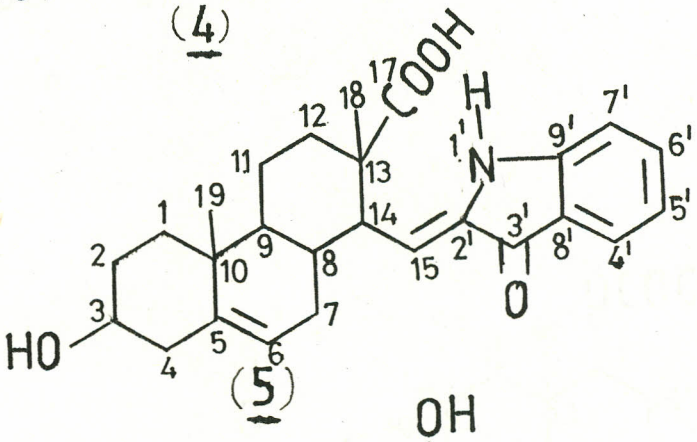

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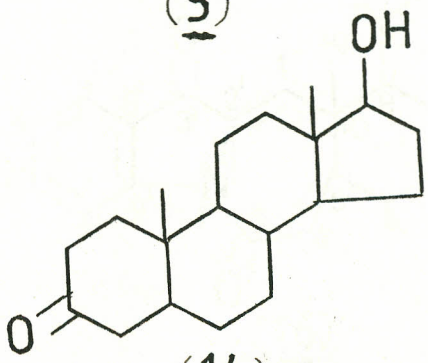

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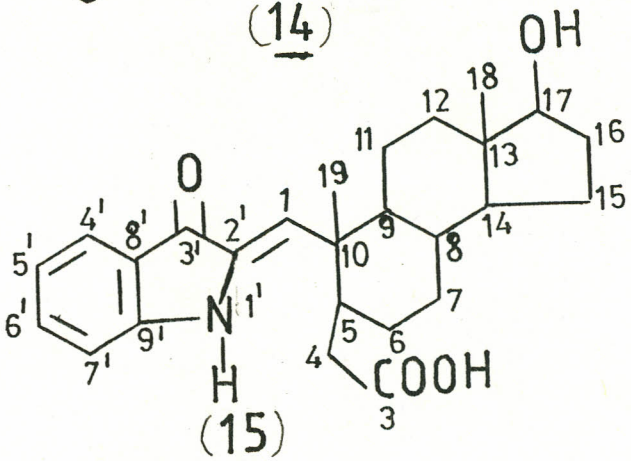

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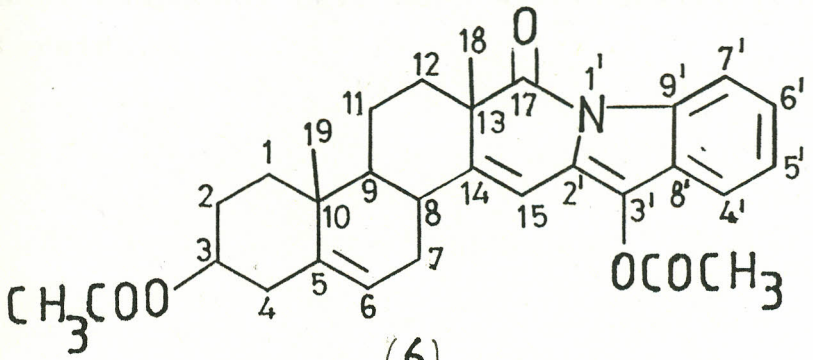
O
18
12
11
13
17
1'
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7'
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1
2
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10
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7
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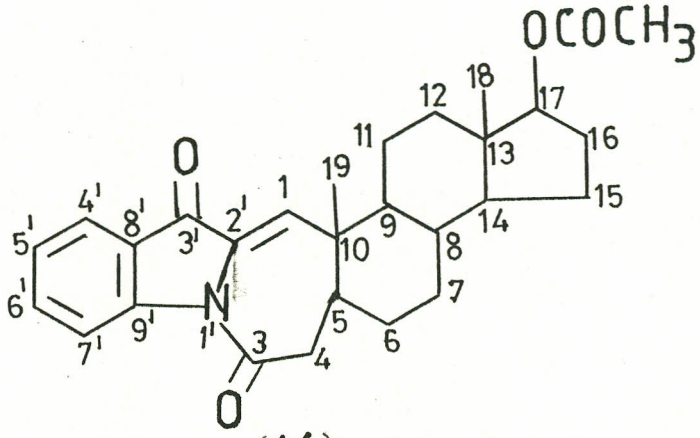
4
CH3COO
OCOCH3

(6)


OCOCH3
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(16)

and Mass spectroscopy where in most cases the model compounds have been structurally related to steroids.

ABSTRACT

3β-hydroxy-5-androsten-17-one (4) condensed in alkaline conditions with 2-nitrobenzaldehyde to give a steroidal indoxylidene carboxylic acid (5), which on refluxing in acetic anhydride gave a 3,3'-diacetyl derivative (6). Reduction of (5) with sodium borohydride in ethanol gave the indole (7) which was cyclised to the lactam (8) by refluxing in acetic anhydride. 3β-hydroxyandrostan-17-one (9) gave the steroidal indoxylidene carboxylic acid (10) on treatment with 2-nitrobenzaldehyde in alkaline conditions. Compound (10) cyclised to the lactam (11) on heating with acetic anhydride and was also reduced to the indole (12) by sodium borohydride in ethanol. Compound (12) was cyclised to the lactam (13) on refluxing in acetic anhydride.

A number of 3-ketosteroids condensed with 2-nitrobenzaldehyde to give a novel group of steroidal indoxylidene carboxylic acids. Thus 5α-androstan-17β-ol-3-one (14), gave the acid (15), which cyclised to the lactam (16) on refluxing in acetic anhydride. It was also reduced to the indole (17) by sodium borohydride in ethanol. The steroidal indoxylidene carboxylic acid (19), synthesised from 17α-methylandrostan-17β-ol-3-one (18), was also reduced to the indole (21). Other 3-ketosteroids which gave indoxylidene carboxylic acids were 5α-pregnan-3,20-dione (22), which gave (23) together with an impurity which was tentatively identified as the 21-(o-nitrobenzal) derivative of (23), and 5α-cholestan-3-one (24), which gave (25). 5'-Chloro substituted indoxylidene acids (20) and (26) were also synthesised from (18)

and (20) respectively, by reaction with 5-chloro-2-nitrobenzaldehyde.

Compounds (5),(7),(8),(10),(11),(12) and (13) have been synthesised previously, while the rest are new compounds.

The structures of the synthesised compounds were determined variously from spectroscopic and elementary analysis data, and from literature data for compounds whose synthesis has been reported previously, as well as from reduction and cyclisation reactions to the expected indoles and lactams respectively.

The synthesised compounds were screened for anti-inflammatory activity by the inhibition of carrageenan - induced rat paw oedema method at an initial oral dose of 50mg/kg. The only active compounds at this dose level were the steroidal indoxylidene carboxylic acids (5),(20),(23),(25) and (26). The ED_{50} for the two most active compounds (5) and (25) were determined and found to be 4.79 and 1.99g/kg respectively. The ED_{50} for indomethacin and dexamethasone, included for comparison, were found to be 5.01 and 56.23mg/kg respectively. The log-dose response curves for (5) and (25) were parallel to each other, but not to those of indomethacin or dexamethasone, which precluded a direct comparison of potency between compounds (5) and (25) on the one hand and indomethacin and dexamethasone on the other, and which probably suggest a different mechanism of action from indomethacin and dexamethasone.

The pKa and log P(octanol) values of some of the synthesised acids were determined for

comparison with similar values reported for non-steroidal and steroidal anti-inflammatory drugs. The pKa values found were considerably higher than those reported for acidic non-steroidal anti-inflammatory drugs, indicating that the synthesised compounds are relatively weak acids. The log P octanol values were likewise much higher than those reported for acidic non-steroidal anti-inflammatory drugs. Those of the active compounds (23), (25) and (26) were much higher than log P (octanol) values reported for anti-inflammatory steroids. The results also show that for the homologous series (15), (19), (23), (25) and (26) derived from 3-ketosteroids, there is a progressive increase in anti-inflammatory activity as the log P value increases. In this respect these compounds resemble the anti-inflammatory steroids.

Finally, the results indicate that a chloro substituent on the aromatic ring, as with acidic aryl anti-inflammatory drugs, has a positive effect on activity, since (20), the chloro analogue of (19) was active while compound (19) itself was inactive. Compound (26), the chloro analogue of (25), was more active than the latter.

CHAPTER I

INTRODUCTION

a) general

The search for compounds with desirable biological activity profiles is a perpetual challenge in medicinal chemistry. The discovery process of such compounds can be divided into two general approaches:

(1) the attempt to find new 'lead' compounds

(2) the attempt to fully exploit existing 'lead' compounds.

A 'lead' compound in this context is a molecule that has the biological activity of interest, although the activity may be weak, may have side effects, or may be non-selective.

The search for new 'lead' compounds can proceed in any one or a combination of the following ways:

1) isolation, purification and identification of compounds from natural products. Examples of bioactive compounds found in this way include antibiotics, alkaloids, steroids and cardiac glycosides

2) following up leads generated by therapeutic folklore or folk medicines

3) testing of metabolites or molecular modifications of metabolites of known compounds

4) fundamental studies of biochemical systems
5) investigation of side effects of experimental or clinically used compounds
6) mass screeening of chemical compounds for possible biological activity
7) organic syntheses aimed at producing novel bioactive compounds.

The next step in the discovery of bioactive compounds is the pursuit of the 'lead' compounds. This pursuit permits the employment of structure-activity relationships. The procedures for rationally exploiting a 'lead' are much more fully developed than those for the development of new leads, as is reflected in the voluminous literature pertaining to the former. The search for new biologically active compounds therefore contains elements of both expiricism and design.

The rational pursuit of a 'lead' compound requires a knowledge of the relationship between structure and biological activity. Studies aimed at the further exploitation of a 'lead' compound consist in manipulating the structure of the compound. The basic assumption in such manipulations is that structurally similar compounds have similar action. Thus small perturbations in structure should result in small perturbations in biological activity. For example, conversion of morphine to its 6-acetyl derivative leads to improvement in its narcotic analgesic potency.

Through systematic alteration of the structure of the active molecule, a more desirable molecule may be formed. Unfortunately, the number of possible alterations for even a simple compound is often astronomical. Therefore, in medicinal chemistry, one is faced with the prospect of judging which few of a large number of possible compounds should be synthesized. A further complication lies in the fact that structural similarity is not the only factor governing drug action. The biological effectiveness of a molecule is governed by a combination of that molecule's electronic nature, steric nature and transport properties. Structural alterations can have various effects on each of these factors and thus similarity with respect to structure may not be readily apparent. Even so,it remains that alterations in the factors that govern activity are available largely through modifications of structure.

The structure of a compound affects two properties which are closely interrelated in relation to its biological activity. These two properties are the physical characteristics and the chemical reactivity. Physical properties can be decisive in determining whether or not a molecule will show a certain biological activity. Such properties will determine for example whether the molecule can permeate various barriers between the external medium and the site of possible action within the organism, and arrive there in adequate concentrations.

Secondly, there can be no doubt that a high proportion, if not the majority of biologically active compounds owe their activity to a capacity for participating in a chemical reaction or forming some looser combinations of a specific nature with cell constituents. There is a gradation in the firmness of the combination which can be effected between a biologically active molecule and a cell constituent. At the one extreme there are reactions, often irreversible, which result in the formation of covalent bonds. At the other extreme, there are looser unions by electrostatic attraction, Van der Waals forces or hydrogen-bonds. In these cases the extent of reversibility is governed by the forces comprising the union.

The physicochemical characteristics of a compound which owes its biological activity basically to a chemical reactivity will exert an important effect on the activity. The absorption of the compound and its passage through membranes is greatly affected by such properties as solubility, ionic dissociation and stereochemical arrangement. Its access to the site of action will be influenced by these properties. Complete failure of access will result in loss of biological activity.

It follows therefore that a biologically active compound must satisfy at the same time the requirements of chemical reactivity and of physicochemical properties. Only in the area where both sets of properties are within the permissible range will biological activity

ensue. This is one of the reasons why the position and nature of substituents in a complex molecule can have an important and even decisive influence on the biological activity. It probably acccunts for the fact, frequently observed, that only a limited number, perhaps only one, of compounds of a particular chemical class exhibits a characteristic biological activity.

In organic synthetic efforts aimed at producing new biologically active compounds, it is often useful to take account of structural, physicochemical and pharmacological characteristics of agents already available in the therapeutic area of interest, and to speculate in which way these characteristics can be advantageously modified.

b) Pharmacology

The majority of drugs currently in use as anti-inflammatory agents can be divided broadly into steroidal, and non-steroidal anti-inflammatory drugs. Of the non-steroidal anti-inflammatory drugs (NSAID), the acidic type predominates.

The corticosteroids at doses required to control inflammatory conditions, cause a number of side effects, including exacerbations of peptic ulcers (Janowitz, Weinstein, Shaer, Cereghini and Hollander, 1958), lowering of host resistance to infection (David, Grieco and Cushman, 1970; North, 1971), impairment of protein synthesis leading to skeletal muscle wasting (Engel, 1966), convulsive disorders (Janowski, Shaver, Christy and Rosner, 1968), increased bone resorption (Eisenberg, 1964), sodium retention leading to oedema and hypertension (Sprague, Power, Masur, Albert, Mathieson, Hench, Kendall and Slocumb, 1950), and suppression of adrenocorticotrophic hormone (ACTH) release with the associated histopathological changes in the adrenal glands (Myles and Daly, 1974). The ulcerogenic effects of corticosteroids may be grave, especially when there has been a previous history of, or predisposition to ulcers (Nielsen, Drivsholm, Fisher and Brockner-Mortensen, 1963).

The acidic NSAID as a class have a number of undesirable side effects, including damage to the stomach mucosa (Jennings, 1965; Rainsford, 1975; Whitehouse and Rainsford, 1977;

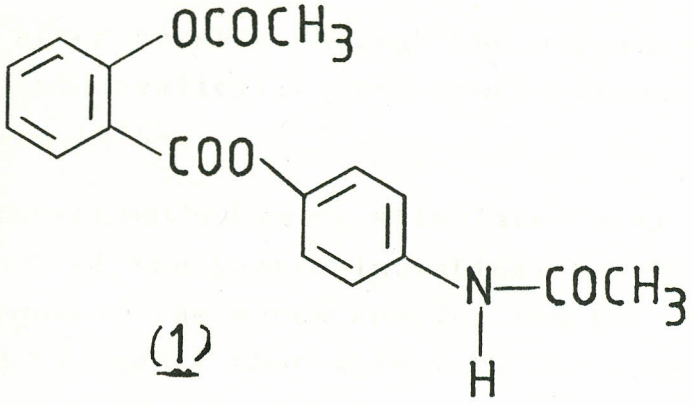
Pemberton and Strand,1979),renal toxicity (Arnold,Collins and Starmer,1976),hepatotoxicity (Hart and Boardman,1965),hypersensitivity(Smith and Smith,1966;) and effects on platelets and blood clotting(Scherrer and Whitehouse,1974). The common side effect of the acidic NSAID is their damage to both gastric and duodenal mucosa (Duggan,1976;Lovgren and Allender,1965; Sun,Roth,Mitchell and Englund,1974). Although the predominant effect of aspirin, for example, is a local one, (on the upper gastro-intestinal tract),under some conditions even parenterally administered aspirin can cause lesions (Barbour and Dickerson, 1938). The binding of aspirin and other acidic NSAID by the blood platelets impairs the aggregation of the latter when brought into contact with exposed collagen (Quick and Clescere,1960),and this facilitates gastric haemorrhage (Schmidt and Green,1972).

Sloughing of both the protective mucus lining the stomach wall and the mucosa cells immediately below the mucosa layer is caused by the high acidity of the drug itself as well as by coagulation of the mucus in contact with the drug (Rainsford,Watkins and Smith,1968). Selective damage to the parietal cells is due to a combination of any ionised drug that is in solution within the gastric pits with protons that are being continually secreted into the pits by the parietal cells that line the pits (Rainsford,1972). The unionised drug that forms thereby can readily pass into parietal cells, ionise therein, and be effectively trapped inside the cells (Rainsford and Brune,

1976).

A number of solutions have been proposed, and tried, in an attempt to circumvent this gastrotoxicity. One is the development of new drugs that are not potentially ulcerogenic i.e. in the case of NSAID, drugs that are not probably acidic and cannot be metabolised to acids. A second alternative is to develop drugs that, though acidic, are less ulcerogenic than currently available drugs. 2,3-Dihydroxy- and 2,3-diacetoxy-benzoic acids have been found to be less ulcerogenic than the traditional salicylates (Whitehouse,Rainsford, Ardlie,Young and Brune,1976). 4-Nitro-2-phenoxymethane sulphanilide, an acid anti-inflammatory drug that lacks a carboxylic acid, has experimentally been found to be less ulcerogenic than the salicylates(Swingle,Moore, and Grant,1976).

A third alternative is to temporarily remove the acidic (and ulcerogenic) characteristics of the drugs by masking this acidic function. The principle of masking the acidic moiety of acidic NSAID with such moderately labile groups as esters or other derivatives to produce latent forms(pro-drugs) appears a most effective means of reducing interaction of the irritant acidic NSAID in the acidic milieu of the stomach with drug-sensitive mucosa and parietal cells. Benorylate (1) is one such ester of aspirin(Croft,Cuddigan, and Sweetland, 1972). Another example is 7-chloro-3,3α-dihydro -2-methyl -2H,9H - isoxazole-(3,2,1)-benzoxazin -9- one, (2) which is non acidic and breaks down



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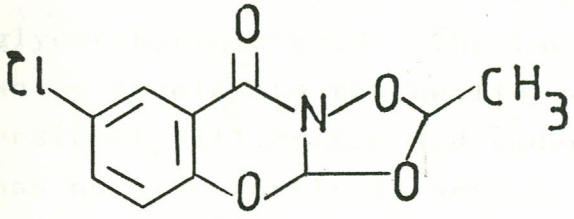

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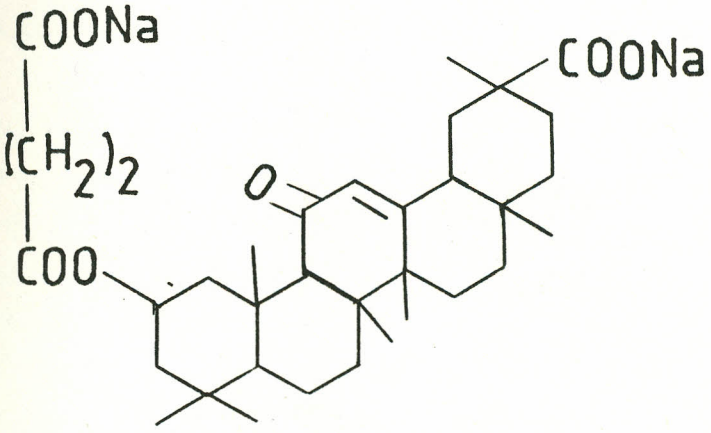


(3)

in vivo after passage through the stomach to yield 5-chlorosalicylic acid (Sofia, Diamantis and Ludwig, 1975).

A fourth method is to stimulate local resistance of the gastro-intestinal tract within the stomach. The mucus coating can be improved by agents that stimulate its biosynthesis.

Carbenoxolone (3) has just such an effect (Shillingford, Lindup and Parke, 1974). Carbenoxolone is the water-soluble derivative of glycyrrhetinic acid. The latter has been shown to accelerate the healing of gastric ulcers (Doll, Hill, Hutton and Underwood 1962). It has no glucocorticoid action, but the compound shows anti-inflammatory activity (D'Arcy and Kellet, 1957).

c) Chemistry

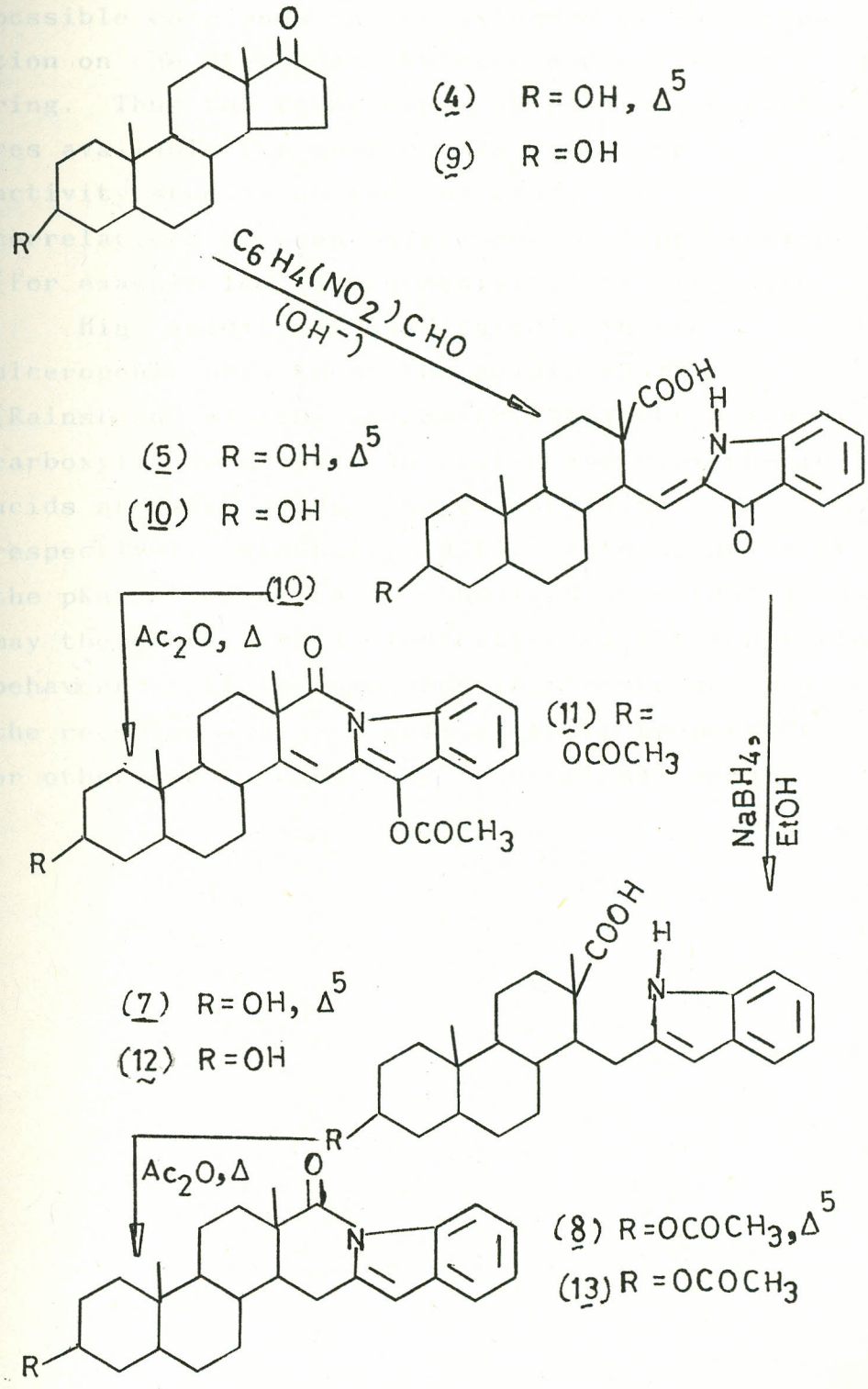
The synthesis of steroidal indoxylidene carboxylic acids of the type (5) and (10), their respective indole (7),(12) and lactam (6),(8),(11),(13) derivatives (Chart 1) was first reported by Hassner, Haddadin and Catsoulacos(1966) in the course of their studies on the synthesis of steroidal nitrogen heterocyles. Thus, treatment of 3β- hydroxy - 5-androsten-17-one(4)or its 5,6 - dihydro analogue(9)with 2-nitrobenzaldehyde in alkaline conditions yielded, respectively, (5), and (10). Compound (10) could be converted on heating with acetic anhydride to the lactam (11). The indoles (7) and (12) were synthesized by reduction of the indoxyls (5) and (10) respectively, with sodium borohydride in alcohol. When the indoles (7) and (12) were heated with acetic anhydride, they gave the lactams (8) and (13) respectively.

Pharmacological studies on compounds of this type have not been reported in the literature. Structurally, these compounds bear some features of both steroids and acidic NSAID, insofar as they possess a partially opened steroidal skeleton as well as having a free (indoxyls and indoles)or masked (lactams) carboxylic acid moiety and a heteroaryl ring system. These compounds are therefore suitable candidates for screening as potential anti-inflammatory agents.

The synthesis of these compounds from 17-ketosteroids involves a 3-step synthesis for the indole lactams. From a synthetic view point therefore, these compounds are easily accessible,

CHART 1

Synthetic pathways for steroidal indoxyls, indoles and lactams from 17-keto steroids (Hassner et al 1966)



and they also offer a variety of structures, namely the indoxyl and indole acids, as well as their derived lactams, all of which, from a structural point of view, are suitable candidates for screening as potential anti-inflammatory agents. Besides, the number of possible compounds can be extended by substitution on the steroidal skeleton and/or aromatic ring. Thus the total range of possible structures available for qualitative structure - activity studies on the one hand, and correlations between physicochemical properties (for example log P) and activity, is extensive.

High acidity is associated with the ulcerogenic effects of the acidic NSAID (Rainsford, Watkins and Smith,1968). Steroidal carboxylic acids such as cholic and deoxycholic acids are weak acids, pKa 6.4 and 6.58 respectively,(Windholz, 1976). An evaluation of the pKa values of the synthesized steroidal acids may therefore give an indication of the ionisation behaviour of these compounds in the stomach and at the receptor site, as well as their propensity or otherwise to cause gastrointestinal damage.

d) Aim of the present work

The aim of the present study was therefore:

(i) The synthesis of some steroidal indoxylidene carboxylic acids, their reduced indole acid derivatives, and the cyclic lactam derivatives of these acids.

(ii) The screening of synthesised compounds for anti-inflammatory activity to determine if active 'lead' compounds could be thus uncovered.

(iii) The attempt at correlation between structure, physicochemical properties and activity for the screened compounds.

Experiments were therefore designed with the following objectives:

(1) To repeat the synthesis of some of the compounds reported by Hassner et al (1966) (Chart 1).

(2) To find out if similar steroidal carboxylic acids (15), (19), (20), (23), (25) and (26) could be synthesized from 3-ketosteroids.

(3) To attempt the synthesis of steroidal indole carboxylic acids (17) and (21) from (15) and (19) respectively (Chart 2).

(4) To attempt the synthesis of the lactam (16) from (15) (Chart 2).

(5) To determine if any of the synthesized compounds show anti-inflammatory activity in animal models.

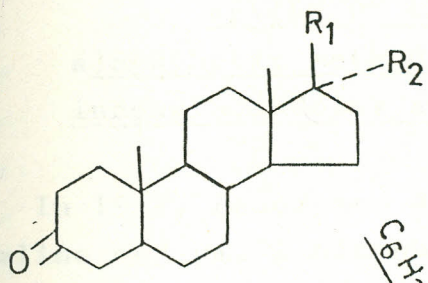
(6) To establish if there is a qualitative correlation between structure and activity for these compounds.

(7) a) To find out if the synthesized acids satisfy the physicochemical criteria as established for the acidic NSAID, namely pKa valued 4-5 and log P(1-octanol) of 0-1.

b) To establish if there is a correlation between pKa, log P(1-octanol) and activity for the synthesized compounds.

CHART 2

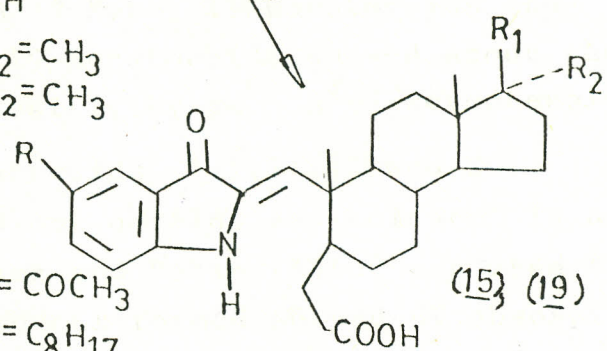
Synthetic pathways for proposed steroidal indoxyls, indoles and lactams from 3-ketosteroids



(14) $R_1 = OH, R_2 = H$
(18) $R_1 = OH, R_2 = CH_3$
(22) $R_1 = COCH_3, R_2 = H$
(24) $R_1 = C_8H_{17}, R_2 = H$

$C_6H_3(R)(NO_2)CHO$
(OH^-)

(15) $R = R_2 = H, R_1 = OH$
(19) $R = H, R_1 = OH, R_2 = CH_3$
(20) $R = Cl, R_1 = OH, R_2 = CH_3$

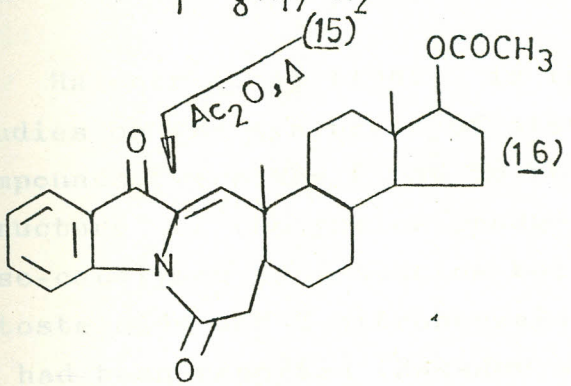


(23) $R = R_2 = H$ $R_1 = COCH_3$
(25) $R = R_2 = H$ $R_1 = C_8H_{17}$
(26) $R = Cl$ $R_1 = C_8H_{17}$ $R_2 = H$

(15), (19)

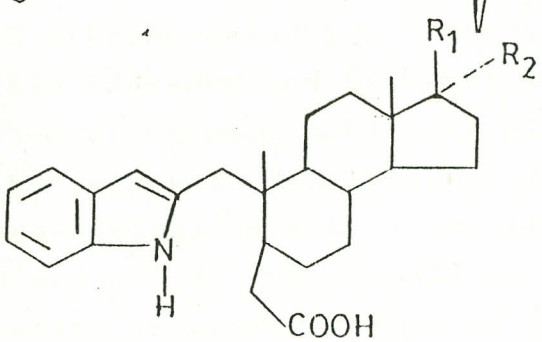
(15)

Ac_2O, Δ



(16)

$NaBH_4/EtOH$



(17) $R_1 = OH, R_2 = H$
(21) $R_1 = OH, R_2 = CH_3$

CHAPTER II

REVIEW OF LITERATURE

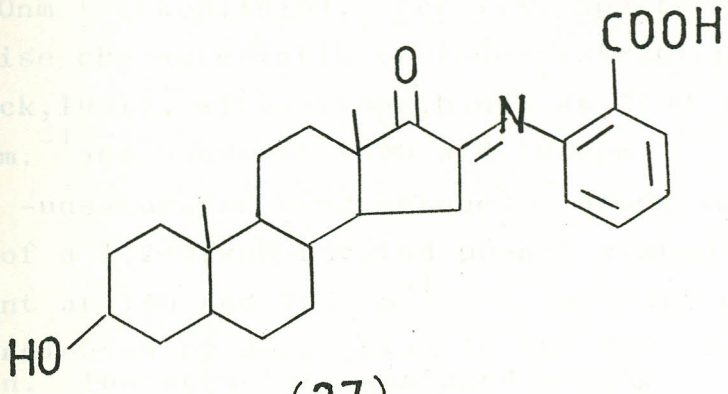
a) Synthetic methods for steroidal indoxyls, indoles and lactams

In 1948, Pesez and Herbain reported that in alkaline medium, 2-nitrobenzaldehyde develops a yellow colour with 3- and 17- ketosteroids. They found the order of sensitivity for this reaction with respect to ketosteroids to be 17-ketosteroids > 3-ketosteroids saturated in ring A> Δ^4- 3-ketosteroids. Δ^4-3-keto, 19-norsteroids and $\Delta^{5(10)}$-3-keto,19-norsteroids showed about the same order of sensitivity as Δ^4-3-ketosteroids. $\Delta^{1,4}$-3-ketosteroids, 6,7,11,12 and 20 ketosteroids did not develop any colcur. In a later paper Pesez and Robin (1962) reported that the coloured products formed showed UV absorption in the 410-460nm range.

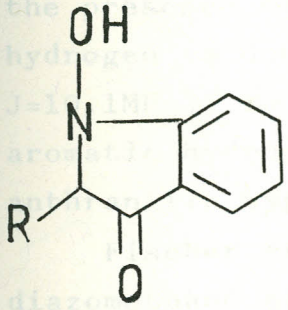
Hassner *et al* (1966), in the course of studies on the synthesis of steroidal nitrogen compounds, were the first to report on the structure of the yellow products formed in the base-catalysed condensation between 17-ketosteroids and 2-nitrobenzaldehyde. Previously, it had been reported (Hassner and Cromwell;1958) that basic media are often unsuitable for aldol condensations of ketones with 2-nitrobenzaldehyde, and that such condensations were best performed in sulphuric acid media. Acidic conditions were found ineffective in condensations of 3β - hydroxy -5- androsten -17- one with 2-nitrobenzaldehyde (Weigert and Kummerer,1913). In alkaline

conditions, Hassner et al (1966) reported that the expected 2-nitrobenzal ketone was not formed with 3β -hydroxy -5- androsten -17- one, but they were able to isolate, in good yield, a yellow acidic material showing no absorption in the I.R. for a nitro group. The possible structure (27), formed by photolytic conversion of 2-nitrobenzaldehyde to 2-nitrosobenzoic acid and then reaction with the 17-ketosteroid, was discarded for the product of condensation since the ketone did not react with 2-nitrosobenzoic acid. The ketone reacted, even in the dark, with 2-nitrobenzaldehyde. The U.V. spectrum of the product indicated a highly conjugated system, λmax 238, 262 and 455nm. The acid formed a brown 2,4-dinitrophenylhydrazone at the 3'-keto group. It also displayed flourescence in some organic solvents.

The partial structures (28) and (29), where R is the steroidal moiety, though mechanistically attractive, were eliminated, the former on the basis of elementry analysis, colour and acetylation experiments, the latter on the basis of the IR spectrum (presence of -NH absorption). The long wavelength λmax in the UV of the yellow acidic material showed a bathochromic shift with respect to the reported λmax for indoxyls,



(27)



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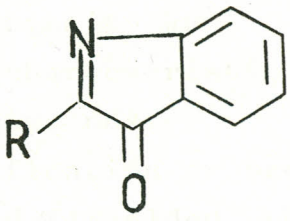
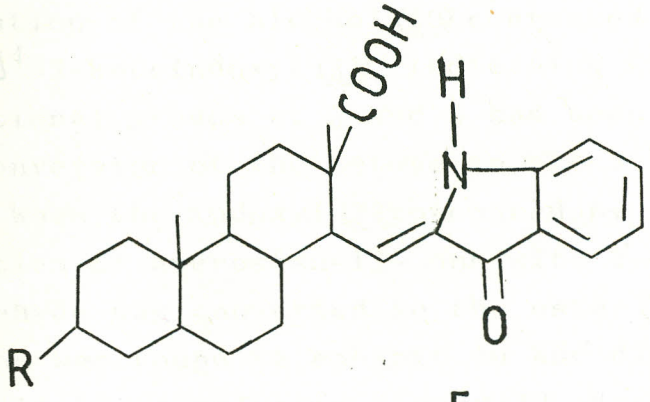


(29)



(5) R = OH, Δ^5

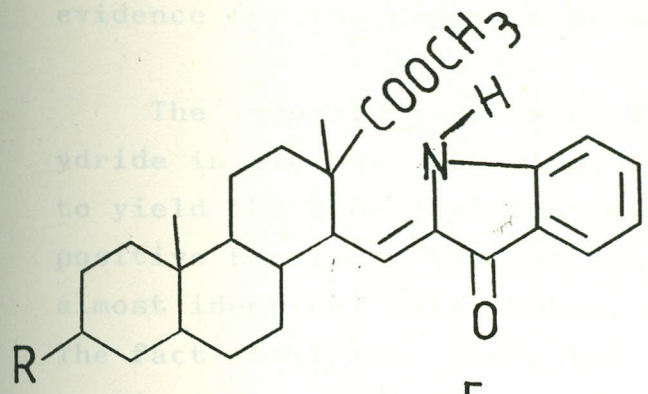
(10) R = OH

of 400nm (Witkop,1950), The I.R. Spectrum was likewise characteristic of indoxyls(Witkop and Patrick,1951), with strong bands at 3400 and 1600cm.$^{-1}$and bands at 1690 and 1640cm.$^{-1}$ (α,β -unsaturated ring ketone). Bands indicative of a 1,2-disubstituted phenyl system were present at 750 and 705 cm^{-1}. A carboxyl group was indicated by absorption in the 2400-2750cm^{1} region. The structure assigned was (5).

The analogous acid (10), formed by 3 β -hydroxyandrostan-17-one, showed in the NMR spectrum the presence of an alkenic hydrogen split by one hydrogen (a doublet at δ 5.77, J=10.1MH$_z$), in addition to an -NH signal and aromatic hydrogens characteristic of indoxyls or anthranilic type compounds.

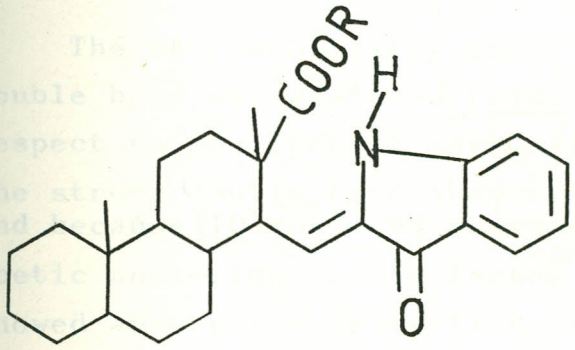
Fischer esterification or treatment with diazomethane of acid (5) yielded a methyl ester (30), which could be acetylated at room temperature to the corresponding 3- acetate. Oppenauer oxidation of the alcohol (30) converted it into a Δ^4-3-ketoindoxyl (31), indicating that the functional groups at 3-and 5-had been unaffected by conversion of the ketone to the acid (5).

When the indoxyl (32), prepared by analogous reaction of androstan-17- one with 2-nitrobenzaldehyde was converted to the ester (33), the latter was found to exhibit in KBr disc as well as chloroform solution strong-NH absorption at 3350cm.$^{-1}$, indoxyl carbonyl and methyl ester carbonyl absorption at 1700 and 1730cm^{-1}, respectively, absorption at 1640(conjugated C=C) and N.M.R. doublet at δ 5.77, all consistent with structure (33).



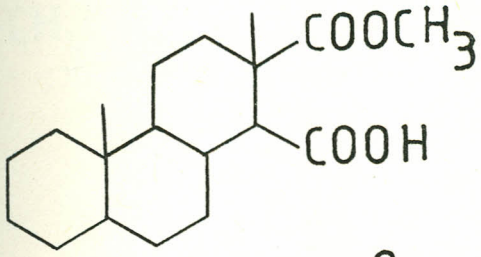
(30) R = OH, Δ^5

(31) R = O , Δ^4

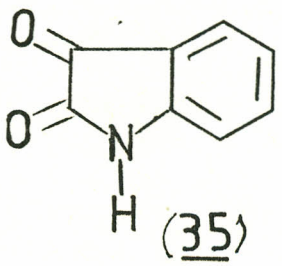


(32) R = H

(33) R = CH_3



(34)

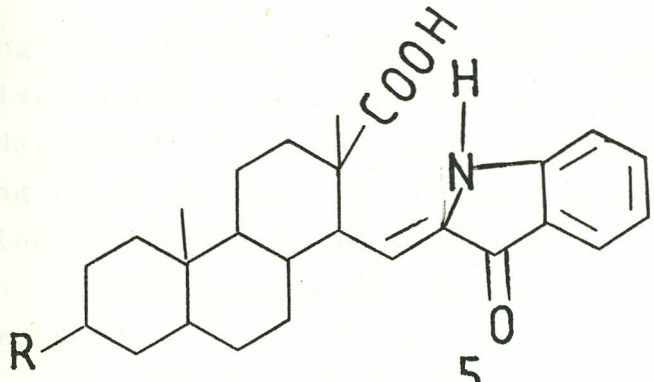


(35)

Oxidation of the ester (33) with chromic acid gave (34) and isatin (35), thus providing additional evidence for the presence of an indoxyl nucleus.

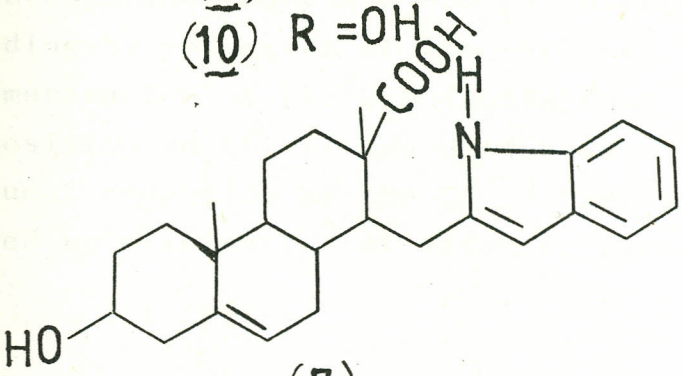
The indoxyl (5) was reduced by sodium borohydride in alcohol with simultaneous loss of water to yield the steroidal indole (7), which gave a positive Ehrlisch test and had a U.V. spectrum almost identical with that of 2-methylindole. The fact that (7) was converted by heating with acetic anhydride to lactam (8), with no -NH, COOH or anhydride bands in the I.R. also implied a 2-, rather than 3- substituted indole structure.

The stereochemistry about the indoxylidene double bond was assigned trans (steroid with respect to the indoxyl carbonyl) on the basis of the strong double bond absorption at 1640 cm^{-1} and because (10) could be converted on heating with acetic anhydride to the lactam (11). The lactam showed absorption in the I.R. at 1770 (C=O of enol acetate), 1725 (C=O of 3-acetate), 1690 (C=O of lactam), 1640; 1240 (C-O of acetate) and 1190 cm^{-1} (C-O of enol acetate).

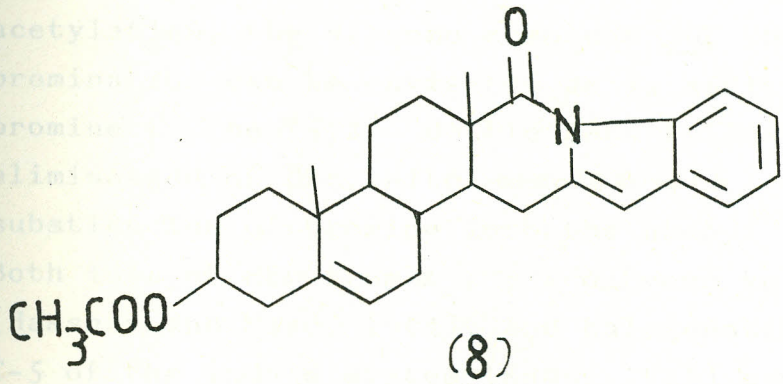


(5) R=OH, Δ^5

(10) R =OH



(7)



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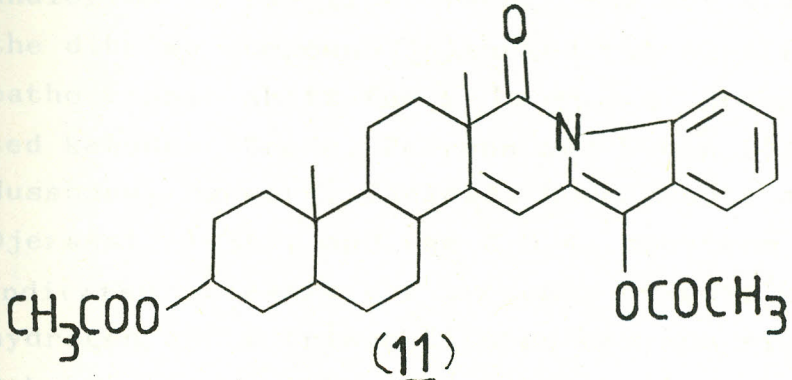
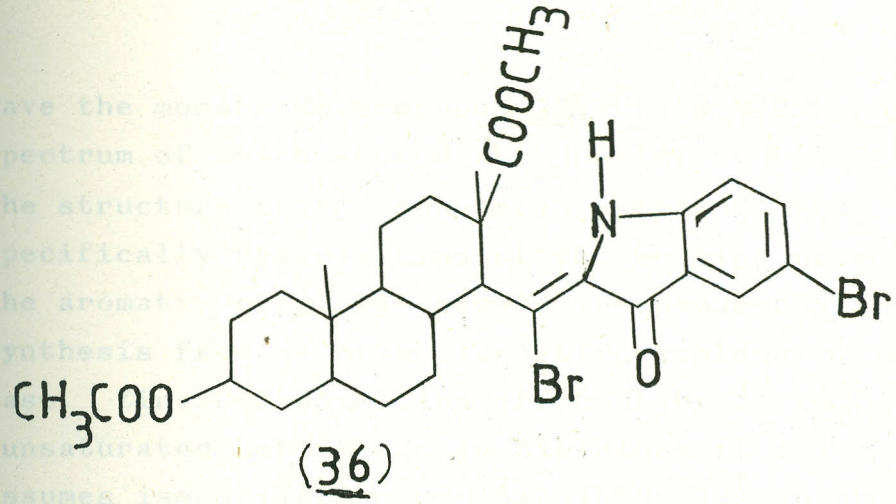


(11)

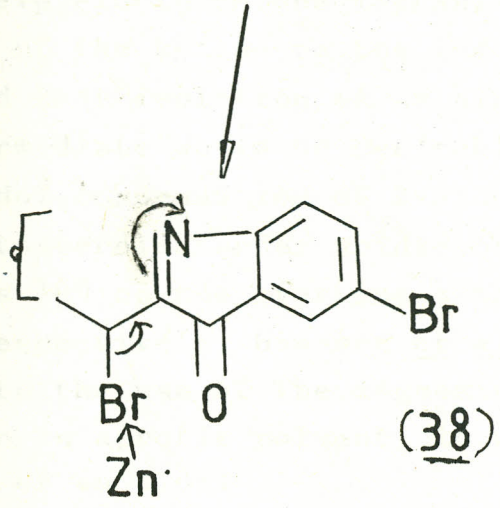
Ring closure of (10) to (11) probably proceeds via a mixed anhydride of 17- oic acid with acetic anhydride, which then acts as an internal acylating agent on the -NH of the indoxyl. The conversion of 17- oic acids to mixed anhydrides has been reported previously (Hassner and Pomeranz, 1962).

Borohydride reduction can be interpreted as proceeding by reduction of the carbonyl followed by isomerization of the exo double bond to the endo position in the presence of base. Subsequent reduction of the C = N bond would be followed by elimination of water to furnish the indole.

The methyl ester derived from acid (10) reacted readily with bromine to yield, after acetylation, the dibromo compound (36). The bromination can be envisaged as an addition of bromine to the 15,2' - double bond followed by elimination of HBr, with simultaneous substitution of bromine into the phenyl ring. Both loss of HBr from α , β - dibromo ketones (Hassner and Mead, 1964), and halogenation at C-5 of the indole system (Kambi, 1941) have analogies in the literature. The U.V. spectrum of the dibromo compound (36) showed the expected bathochromic shift for β - bromo -α , β -unsaturated ketones (Brode, Pearson and Wyman, 1954; Nussbaum, Mancers, Daniels, Rosenkrautz and Djerassi, 1951), and the N.M.R. spectrum indicated clearly the presence of an alkenic hydrogen and a trisubstituted benzene ring. Debromination with zinc in ethereal acetic acid

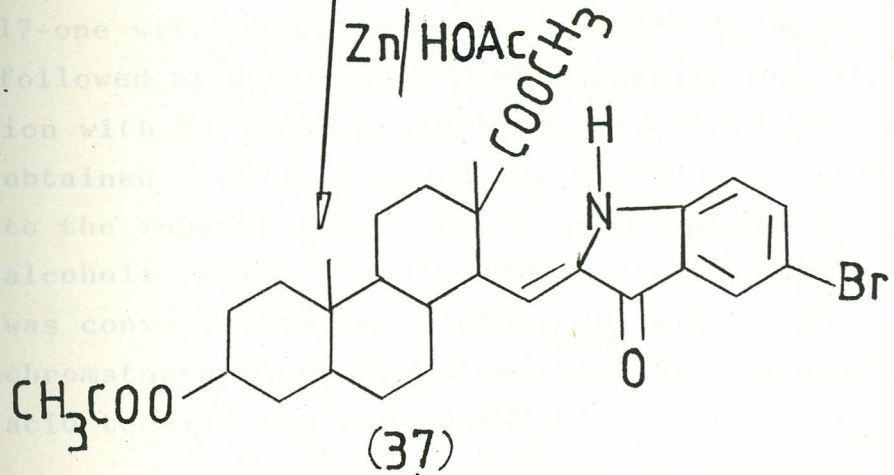


(36)



(38)

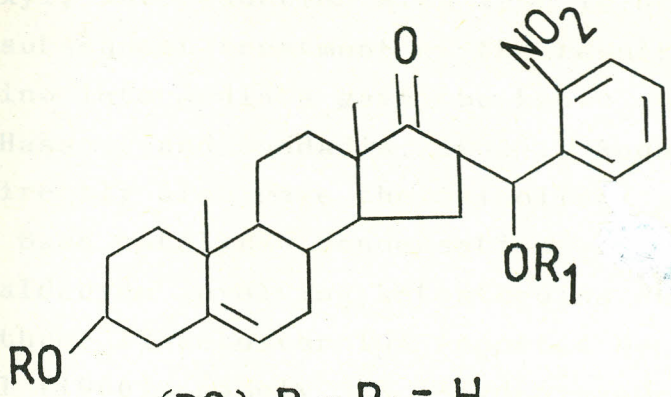
Zn/HOAc



(37)

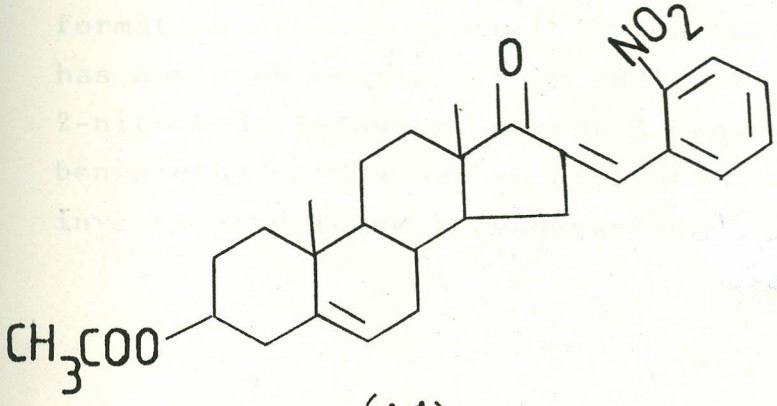
gave the monobromo compound (37), the N.M.R. spectrum of which showed the doublet at δ 5.77. The structure of the monobromo compound, and specifically the position of the bromine atom in the aromatic ring, was proved independently by synthesis from 5- bromo -2-nitrobenzaldehyde in base. Zinc debromination of the β-bromo -α,β -unsaturated ketone can be explained if one assumes isomerization of (36) to (38). (38) can then be reduced by zinc, like an α-bromo -ketone.

To help elucidate the mechanism of transformation of the ketone to the indoxyl, it was considered that isolation of an aldol condensation intermediate would be desirable. Since direct aldol condensation of 2-nitrobenzaldehyde and 17-ketosteroids under acidic or basic conditions led to the starting material or the indoxyl respectively, Hassner et al (1966) resorted to the use of the magnesium enolate of the ketone in aprotic solvent, in which case isolation of an aldol intermediate, owing to the formation of the magnesium salt, would become possible (Collmann, 1961). On treating 3β - hydroxy -5- androsten-17-one with phenylmagnesium bromide in ether, followed by treatment of the magnesium enolate ion with 2-nitrobenzaldehyde, the ketol (39) was obtained. This compound was rapidly converted to the indoxyl 5 on contact with aqueous or alcoholic base. As structural proof of (39), it was converted to the acetate (40), which upon chromatography on alumina (neutral) lost acetic acid to give the compound (41).

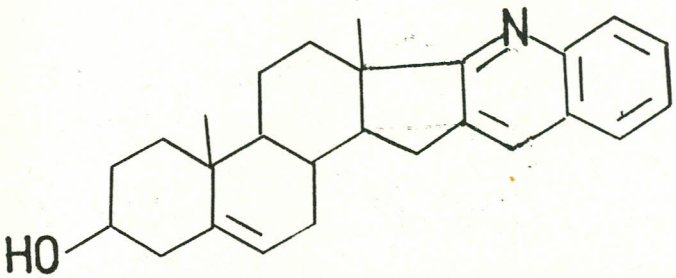


(39) $R = R_1 = H$

(40) $R = R_1 = COCH_3$



(41)



(42)

Treatment of (41) with base did not yield indoxyl, but reduction with iron in acetic acid and subsequent treatment of the resulting anilino intermediate gave the known quinoline (42) (Hassner and Haddadin, 1962). Reduction of (39) directly also gave the quinoline.

Base catalysed condensations with 2-nitrobenzaldehyde involving ketosteroids other than the three 17-ketosteroids reported by Hassner et al (1966), namely 3 β -hydroxyandrostan -17-one, 3 β -hydroxy-5-androsten - 17 -one and androstan-17-one, have not been reported in the literature.

The report by Pesez and Herbain (1948) that 3-ketosteroids among others develop a yellow colour with 2-nitrobenzaldehyde indicates formation of some product, the nature of which has not been examined. In addition, substituted 2-nitrobenzaldehydes, except 5-bromo-2-nitrobenzaldehyde (Hassner et al,1966), have not been investigated in such condensations.

b) The Carrageenan assay for anti-inflammatory activity

The complexity of the inflammatory process and the diversity of the drugs that have been found to be effective in modifying this process have resulted in the development of numerous methods of assay capable of detecting anti-inflammatory substances. A few of these methods have achieved popularity because of their simplicity, economic feasibility and ability to select drugs known to afford some benefit in the clinical management of rheumatoid diseases.

Methods based on the inhibition of an induced swelling have been amongst the most popular in the screening for new anti-inflammatory substances. The general procedure is to inject a small amount of suspension or solution of an oedemagen into the plantar tissue of the hind-paw of the rat. Assessment of the response is usually made at the time of maximum swelling. Of the many oedemagens that have been employed, carrageenan has apparently replaced formalin as the most widely used.

Carrageenan is a mixture of polysaccharides composed of sulphated galactose units and is derived from Irish sea moss, Chondrus crispus (Smith, O'Neill and Perkin, 1955). Its biological properties have been reviewed by Di Rosa (1972). The first report of its use in causing fibrous tissue formation seems to be that of Robertson and Schwartz(1953), but Winter, Risley and Nuss (1962) were the first to apply it to the acute and easily quantifiable oedema. The carrageenan

assay, as usually carried out, is slightly modified from that of Winter et al (1962). The compound to be assayed is given orally in saline or 1% methylcellulose suspension. One hour later, 0.10 ml. of carrageenan suspension is injected into the plantar tissue of the hind-paw and the paw volume recorded .3 hours later, the volume is again recorded. The swelling in the treated rats is calculated as a % of that of the control rats. The methods currently used in the measurement of paw volume are based on mercury displacement as described by Winter et al (1962) or Van Aman, Begamy, Miller and Pless (1965) with automatic recording devices where large amounts of data are required in screening operations. Data are most often expressed as % inhibition of oedema $= 100 \left(1 - \frac{\Delta VT}{\Delta VC}\right)$ where ΔVT and ΔVC are the increases in volume of the carrageenan injected paws of the drug-treated and control group, respectively. Winder, Was and Been (1957) have pointed out that there is no apparent right or left 'footedness' in rats and that paw volumes (non-oedematous) to body weight ratio is constant among rats. Niemegeers, Verbruggen and Janssen(1964) have found no correlation between the volumes of paws before and 3 hours after injection of carrageenan in Wistar rats weighing 185-205 g. A plot of log dose vs % inhibition of the oedema has yielded linear and parallel regression lines for standard anti-inflammatory drugs for some workers(Winter et al ,1962; Swingle, Harrington, Hamilton and Kwam, 1971; Roskowski, Rooks, Tomolonis and Miller,1971) but not for others (Walz,Dimartino,Griffin and Misher,

1970;Green, Green,Murray, and Wilson, 1971). Those obtaining parallel regression lines have been able to use the assay for the comparative bioassay of drugs (Winter, 1966). The failure of some workers to obtain consistently reproducible results with this assay may be due to a variety of causes, but Green et al (1971) attributed daily variations in the response of the oedema to a constant dose of phenylbutazone in part to difference in ambient temperature.

Swelling of the rat's paw after injection of carrageenan is not normally distributed. Van Arman et al (1965) studied the kinetics of the swelling of the paw induced by injection of carrageenan. The development of the oedema in the paw of the rat after the injection of carrageenan has been described as a biphasic event (Vinegar, Schreiber and Hugo,1969). The existence of two phases of swelling probably explains the failure of standard anti-inflammtory drugs to effect a complete inhibition of the oedema. Thus Vinegar et al (1969) have found that it is the second phase of the oedema that is sensitive to such drugs as hydrocortisone, phenylbutazone, and indomethacin. Different investigators have reported maximal inhibition of the oedema induced by carrageenan of 40-80% with standard anti-inflammatory drugs. The evaluation of drugs against the second phase of the oedema seems to be a more sensitive assay for this type of drugs.

The initial phase of the oedema has been attributed to the release of histamine and serotonin; the oedema maintained during the plateau phase, to kinin-like substances; and the second accelerating phase of swelling to the release of prostaglandin-like substances (DiRosa and Willoughby, 1971;DiRosa,Giroud and Willoughby,1971). The recognition of different mediators for different phases of the oedema has important implications for interpreting the effects of drugs. One would not normally expect antagonistic activities to all these mediators to reside in any one drug molecule. It should be pointed out that the identity of these mediators which are responsible for the evolution of the oedema induced by carrageenan, is by no means finally established.

The histamine-serotonin antagonist cyproheptadine has been reported to be ineffective in this assay (Winter, 1966; Vinegar et al,1969). However, pre-treatment of rats with the amine depleter, compound 48/80,abolishes the early phase of the oedema (DiRosa et al,1971), and monoamine oxidase inhibitors potentiate the oedema (Fekete and Kurti,1970). DiRosa et al (1971) have been able to antagonise the early part of the oedematous response with an antihistamine plus cyproheptadine. Crunkhorn and Meacock (1971) felt that serotonin and kinins were involved, especially in the early phase of carrageenan - induced oedema. The involvement of kinin-like substances in the response to carrageenan is suggested by the data of Van Arman et al (1965), and DiRosa and

Sorrentino (1968) who have been able to substantially inhibit the oedema with anti-proteases. Briseid, Arntzein and Dyrud(1971) have correlated changes in the plasma kinin system with inhibition of the oedema. It should be noted, however, that Van Arman and Nuss (1969) concluded that bradykinin is not an essential mediator of carrageenan-induced inflammation. The postulate of involvement of prostagladin-like substances in the second phase of the oedematous response (DiRosa and Willoughby, 1971), may have support in the findings of Willis (1969), who has claimed to extract E-type prostaglandins from the oedema fluid.

In three pioneering papers, non-steroidal anti-inflammatory drugs were shown to inhibit PG biosynthesis in the guinea pig lung homogenate (Vane, 1971), perfused dog spleen (Ferreira, Moncada and Vane, 1971), and in human platelets (Smith and Willis,1971). Vane (1971) has postulated that non-steroidal anti-inflammatory agents exert their pharmacological action through inhibition of PG biosynthesis in tissues. This concept has been supported by numerous experiments, as reviewed by Flower (1974), and Ferreira and Vane (1974).

NSAID inhibit PG biosynthesis at a very early stage of the cyclo-oxygenase activity. Therefore, NSAID also inhibit the formation of cyclic endoperoxides and thromboxanes. Inhibitory action on PG synthesis is a common feature of all acidic NSAID.
However, some non-acidic NSAID eg. indoxole,

have also been reported to inhibit PG synthesis (Ham, 1972). On the other hand, anti-inflammatory steroids are not PG synthetase inhibitors (Flower, 1974). There exists a rough correlation between anti-PG synthetase and anti-inflammatory potencies of NSAID (Ziel and Krupp, 1975). What is more, it has been demonstrated that they inhibit PG synthesis *in vivo* (Collier and Flower, 1971).

The carrageenan bioassay is suited for the comparative bioassay of anti-inflammatory drugs and the relative potency estimates obtained for certain drugs seem to reflect clinical experience. However one must be aware of the limitations of any one method and most workers elect to evaluate potential anti-inflammatory compounds by a 'battery' of test methods. The carrageenan test however has proved to be a reliable method for detecting anti-inflammatory substances. The method is simple, it sheds light on the potency of the substance, and it also conveys an initial impression of the tolerability of the substance, insofar as it is to some extent possible by observing the behaviour of the rats to deduce whether or not side effects occur within the pharmacologically active dose range.

c) Methods for pKa and log P Determinations

Determination of pKa values

The ionisation of an acid involves a protolytic reaction with the base, water. For an acid, such a reaction proceeds according to the equation

$$HA + H_2O \rightleftharpoons A^- + H_3O^+,$$

in which all the entities are hydrated. This equilibrium is denoted by the equation

$$K' = \frac{[a_{A^-}]\,[a_{H_3O^+}]}{[a_{HA}]\,[a_{H_2O}]}$$

With small concentrations of the acid $[a_{H_2O}]$ is virtually constant and the equation above may be restated in the form

$$K = K'[a_{H_2O}] = \frac{[a_{A^-}]\,[a_{H_3O^+}]}{[a_{HA}]} = \frac{[A^-]\,[H_3O^+]}{[HA]}$$

Strictly, K is a function of the acid concentration but experimental errors in K are usually far larger than this effect; hence it suffices, at least for low concentrations, to describe the equilibrium in terms of the virtual constant K.

A number of methods are available for the determination of the ionisation constant K, for example potentiometric, conductance and optical methods, as well as methods based on the partitioning and solubility properties of the acid.

By far the most convenient method for the determination of ionisation constants is potentiometric titration. In this method measurements of pH are made during stepwise titration of a known weight of the acid with standard potassium hydroxide. The mole ratios of the acid-base conjugate pairs is calculated from the amount of titrant added, and the equation $pKa = pH + \log \frac{[HA]}{[A^-]}$ is used.

The most commonly used electrode system is the glass electrode in combination with Standard Calomel Electrode (Albert and Sergeant, 1971).

Potentiometric methods of determining pKa by titration in mixtures of water and water-immiscible solvents have been used. In such systems a set of pKa values is determined in several water-organic solvent mixtures of known composition. A plot of pKa values against % organic solvent in the mixture is performed and extrapolated to zero organic solvent content to get the true pKa value. This method is suitable for compounds which have low solubility in water and has been used in the determination of the pKa of for example morphine(Tencheva,Velinov and Budenvsky,1979).

Determination of pKa by conductance measurements is based on the fact that only free ions can participate in the transport of current through a solution of an electrolyte and hence the degree of dissociation of an electrolyte can be deduced from the observed conductance. The dependence of the equivalent conductance on the total concentration C of an electrolyte may

be stated as

$$\Lambda_C = \Lambda_0 f_\Lambda \alpha$$

Λ_C is the equivalent conductance at concentration C,

Λ_0 is the limiting value of Λ_C at C = 0 and is obtained by extrapolating the measured values of Λ_C at given concentrations C to C = 0, f_Λ is the conductance coefficient which takes into account the effect of interionic attraction, and α is the degree of dissociation.

Conductance measurements thus yield values of the product αf_Λ and if f_Λ is known, it is possible to calculate α and hence K, since $K = \frac{\alpha^2 C_0}{1-\alpha}$

f_Λ can be calculated by successive approximations from the Debye - Huckel - Onsager equation (Davies, 1930; McInnnes, 1926).

In general, the optical properties of an undissociated acid are different from those of its anion A^-. This is true for example for the refraction, fluorescence, Raman effect and light absorption. If it is possible to make use of this difference for the determination of the concentrations of the two forms HA and A^-, then the dissociation constant can be calculated. The most exact values of K are obtained from the different absorption characteristics of HA and A^-(Halban and Ebert,1924). Direct measurement of the degree of dissociation requires the assumption that a wavelength is available at

which the extinction coefficient of one form is negligible as compared to that of the other or that, alternatively, the extinction coefficient at a certain wavelength for HA and A^- are quite different, eg. $\varepsilon_{HA} >> \varepsilon_{A^-}$
If K is not very small, almost no free acid is present in a weakly alkaline solution of the sodium salt of the acid. Measurements of ε of such solutions then give ε_{A^-} and hence α in solutions containing different concentrations of pure acid. The method only requires the assumption that ε_{A^-} is independent of the cation (Na^+ or H_3O+) at the concentrations involved. The applicability of the method to precise determinations of K is therefore restricted to ranges of concentrations in which specific differences in the ionic effects on ε_{A^-} are not to be expected (Kortum, 1935).

Raman Spectroscopy has been used to determine the ionisation constants of strong acids such as nitric acid (Young, Wu and Krawetz, 1957) and weak bases (Deno and Wisotsky, 1963).

Nuclear Magnetic Resonance spectroscopy has proved useful for substances whose U.V. spectra do not change upon ionisation and which are too weak as acids or too poorly soluble. In practice a series of solutions of known pH or acid function are prepared, and the chemical shift of a non-exchanging proton , is plotted against pH. The high concentration at which N.M.R. values must be determined limits the accuracy of the method, but it can usefully define the region in

which the pKa lies, when all other convenient methods fail (Levy,Cargioli and Racella,1970).

The determination of the increase in solubility of an acid at various pH values can be used for the determination of pKa. It is useful when the substance is too insoluble in water. The solubility of an acid depends on two properties; the ionisation constant, and the intrinsic solubility of the neutral molecule. The observed solubility S_0 of an acid at a given pH is due to two terms; the solubility of the neutral molecule (a saturated solution) and the solubility of an anion (far from saturated).

Thus $S_0 = [HA] + [A^-]$

But $[A^-] = \frac{K[HA]}{[H^+]}$

Hence $S_0 = [HA] \quad \frac{K[HA]}{[A^-]}$

$= [HA]\ [1 + \text{Antilog}\ (pH - pKa)]$

In this equation $[HA]$ equals the intrisic solubility S_i i.e. the molar concentration of a saturated solution in which ionisation has been prevented

Thus

$$S_0 = S_i + [1 + \text{antilog}\ (pH - pKa)]$$

The intrinsic solubility of an acid is usually determined in 0.1 Molar hydrochloric acid. The general method as used is as follows : (Krebs and Speakman, 1945), first, the solubility of the neutral molecular species is determined at a pH where it is suspected that this species will predominate. Next, two further determinations are made, 0.5 pH units above and below that formerly used, in order to see whether the same solubility is obtained, a necessary condition to make sure that the original value was the true Si. Then, the solubility is redetermined at a pH near to where the pKa is suspected to be. From these results an approximate value of the pKa is calculated from a rearrangement of the above equation, namely pKa = pH - log(So/Si - 1) for acids. A set of pKa values is then obtained by determining solubilities at a series of pH values preferrably distributed evenly in the range pKa $\pm$ 1.

Partition methods have also been used in the determination of pKa (Farmer and Worth, 1904). The actual partition coefficient C_a of a compound capable of ionising is given by

$$C_a = \frac{C(org)}{C(aq)(1-\alpha)}$$ where

C(org) = concentration of the compound in a

water immiscible organic phase, C(aq)=concentration of the compound in the aqueous phase

α = the degree of ionisation

For acids,

$$\alpha = \frac{1}{1 + \text{antilog}\,(pKa - pH)}$$

The observed partition coefficient, Co, is given by

$$Co = \frac{C(org)}{C(aq)}$$

Therefore

$$Ca = \frac{Co}{1 - \dfrac{1}{1 + \text{antilog}\,(pKa - pH)}}$$

This equation expands to $pH = pKa + \log (Ca - Co) - \log Co$. The general method is similar to the solubility method, except that Co, instead of So, is determined by carrying out partitions at a series of pH values.

From the expanded equation, a plot of log Co against pH gives the pKa at the intercept on the pH axis.

Determination of log P values

The actual partition coefficient P of a compound capable of ionising is given by

$$P = \frac{C(org)}{C(aq)(1-\alpha)}$$

where the terms on the right-hand side of this equation have already been defined. Thus the observed partition coefficient $\frac{C(org)}{C(aq)}$ must be corrected for innisation.

The general method involves dissolving a carefully weighed sample in the phase in which it is most soluble. The calculated second phase is added, the container shaken, then placed in a centrifuge and turned at about 2000 rpm for 1-2 hours. An aliquot of one phase is withdrawn and analysed. The aqueous phase should be saturated with the organic phase before use, and likewise for the organic phase. During the partitioning, if the phases are about equal, equilibrium is rapidly established. Care must be taken so that each phase is still undersaturated. One way of checking this point is to determine the partition coefficients at different concentrations of substance. A constant value indicates that no special interactions of substance are occurring in either phase and that neither phase is saturated.

To minimize errors in calculation the phase should be adjusted in volume so that roughly equal weights of the compound end up in each phase. Sometimes one must deviate from this ideal

situation with molecules of very low or very high P values. Reliable results can be obtained by analysing one phase, but running four separate partitions at different concentrations. The amount of solute found in one phase is then subtracted from the total sample to obtain the amount in the second phase. Standard curves are always run in duplicate, using two different weighings of solute. Compounds with log P values outside ± 3 are most difficult to determine.

d) Structure Activity Relationships Non-Steroidal Anti-Inflammatory Drugs(NSAID)

Although the NSAID have a complex pattern of pharmacological effects, which includes stabilisation of biological membran es (Ignaro,1972; Mizushima and Kobayashi, 1968;), inactivation of proteclytic enzymes (Anderson, Brocklehurst and Willis, 1971), displacement of anti-inflammatory peptides from serum (McArthur, Dawkins and Smith, 1971), and activation of the fibrinolytic system (Gryglewski,1970; Ruegg, Riesterer and Jaques, 1970), the most convincing mode of action of this class of drugs would appear at present to be the inhibitory effects they exert on prostaglandin (PG) synthesis (Vane, 1971). This is suggested, firstly, by the fact that NSAID exercise *in vitro* a concentration-dependent inhibitory action on the enzymatic transformation of arachidonic acid to prostaglandins of the E series. This action is attributable to a complete inactivation of PG synthetase (Flower, 1974). There is also a close correlation between the concentration in which these drugs are active *in vitro* and the potency of their anti-inflammatory and anti-pyretic effect (Ziel and Drupp,1974). What is more, it has also been demonstrated that they inhibit PG synthesis *in vivo* (Collier and Flower, 1971, Smith and Willis, 1971).

Secondly, a further indication of the importance of the role played by PGs in connection with the mechanism of action of the NSAID, is the finding that in the presence of inflammatory processes, local increases occur in PG synthesis (Greaves, Sondergaard and McDonald-Gibson, 1971), and there is more than mere tentative evidence to suggest that the PGs participate as mediators in the pathophysiology of inflammation (Velo, Dunn, Giroud,Timsit and Willoughby,1973), that they are capable of provoking pain(Ferreira, Moncada and Vane,1973), that they contribute to the causation of febrile reaction(Feldberg,Gupta, Milton and Wendlandt,1972; Milton and Wendlandt, 1970), and that they are involved in the phenomenon of platelet aggregation (Smith, Ingerman, Kocsis and Silver, 1973). For these reasons inhibition of PG synthetase has been used as an activity parameter in structure-activity studies of NSAID.

Among the PG synthetase inhibitors, the acidic NSAID, particularly the aryl acids, represent a predominant group. Chemically, they may be divided into several classes: the salicylates, indomethacin analogues, phenylacetic acids, fenamic acids and enolic compounds. Most of them inhibit the cyclo-oxygenase by competing with the substrate arachidonic acid at the active site.

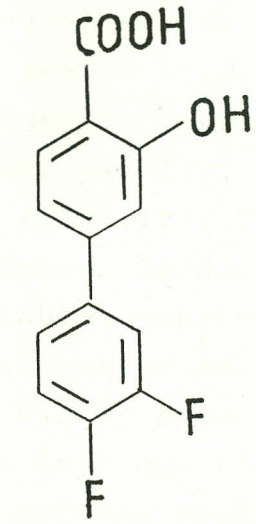
The aromatic moieties in these compounds are sterically and electronically similar to the polyene system in arachidonic acid, possibly interacting with a common binding site. In general, these NSAID possess two non-coplanar hydrophobic or aromatic moieties and an acidic group. The *in vitro* enzyme inhibition and *in vivo* anti-inflammatory activities are often enhanced significantly by electronegative substituents, eg F,Cl, at specific positions in the molecule . Not unexpectedly, there are notable differences in the optimal structural features among the various subclasses. These differences may reflect alternative modes of binding and physicochemical requirements for activity. Similar spatial arrangements of hydrophobic or aromatic moieties are found among the non-acidic PG synthetase inhibitors.

Since the original observation by Vane (1971), that aspirin inhibits PG synthetase, aspirin and other salicylates has been studied extensively. It was reported (Roth, Stanford Majerus, 1975; Rome, Lands Roth and Majerus, 1976), that aspirin quantitatively and selectively acetylates the cyclo-oxygenase at its functional stage. The *in vitro* inhibition by aspirin is about 4 times that of salicylic acid. Replacement of phenolic -OH by -SH or $-OCH_3$ completely abolishes activity. The methyl ester of aspirin is also much less active.

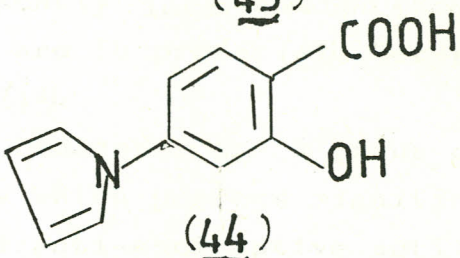
The structural specificity of salicylates has also been demonstrated by examination of a large number of substituted analogues with substituents at 3, 4 and 5-positions. For example, 3-methoxy,

3-chloro,4-phenyl, 5-phenoxy, 5-t-butyl and 5-bromo derivatives are all inactive both as PG synthetase inhibitors and in anti-inflammatory assays *in vivo*. The only exceptions are analogues with a 5-fluorinated phenyl and certain 5-heteroaryl substituents such as diflunisal (43) and the 5-(pyrryl) derivative of salicylic acid (44). In these cases, both the anti-inflammatory and PG synthetase inhibition appear not to be significantly affected by the presence or absence of O-acetyl group (Shen 1979).
Indomethacin (45) is a potent inhibitor of PG synthetase (Vane, 1971) and has been widely used as a reference compound in studies on PG synthetase inhibition and in *in vivo* anti-inflammatory screens. Electronically and stereochemically 1-benzylidenylindene is isosteric with N-benzoyl indole. Sulindac (46) is now used clinically as an anti-rheumatic agent. X-ray crystallographic studies have shown that for the indene series, the configuration of the *cis* isomer is almost identical to that of indomethacin (Hoogsteen and Trenner, 1970; Kistenmacher and Marsh,1972). The *cis* isomer is five times as active as the *trans* isomer in *in vivo* screens (Shen,1979).

The aryl aliphatic acids are a large group of substituted phenyl or heterocyclic aliphatic acids which have been found to possess moderate to potent anti-inflammatory, analgesic and anti-pyretic actions. A number of these have been reported to be PG synthetase inhibitors. The spatial arrangement of the aryl moieties is frequently in accordance with the contours of the hypothetical receptor proposed for



(43)



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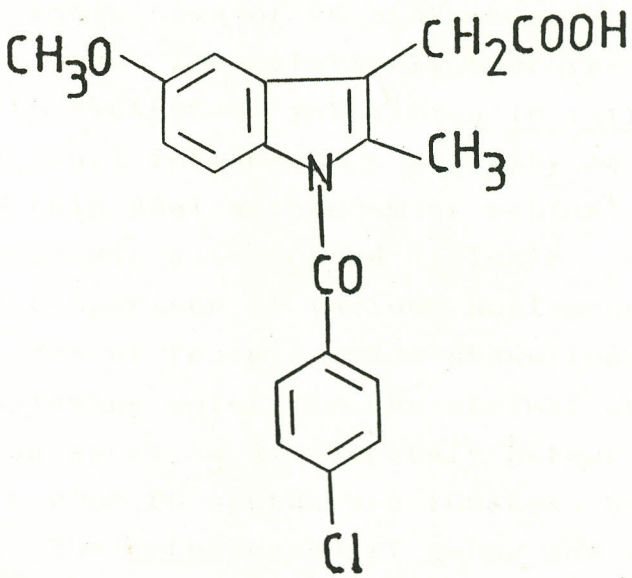


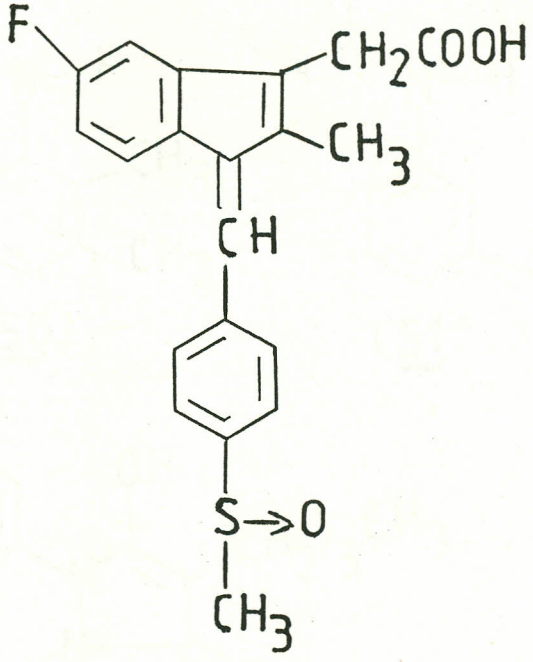
(45)

indomethacin and analogues (Shen, 1977). A notable feature among several of the more potent families is the α— methyl acetic acid side chain with a sinister(S) absolute configuration. The rectus (R) enantiomers are usually, though not always, much less active (Shen,1972;Gaut, Barnth, Randall, Ashley and Paul-Strud, 1976). Most of these acids are chemically stable, substrate-competitive inhibitors of cyclo-oxygenase. The preferred substituents for higher potency vary according to the nature of the aryl moieties in each series, although a preponderance of Cl-or F- substituents are frequently incorporated (Rome and Lands,1975). Examples are Ibuprofen (47), Ketoprofen (48) and Naproxen (49).

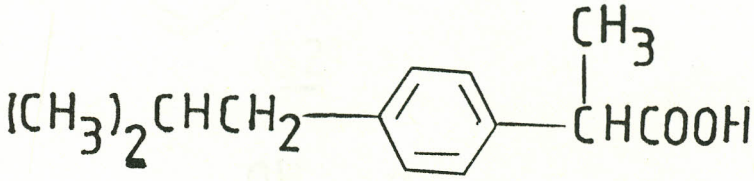
The fenamates are a group of N-Arylanthranilic acids which possess significant anti-inflammatory and anti-nociceptive activities. They are potent inhibitors of PG synthetase as well as PG antagonists. The relatively poor correlation of their PG synthetase inhibition in vitro and their in vivo anti-inflammatory activity may be partly due to this dual mechanism of action. Examples are Mefenamic acid (50) and Niflumic acid (51).

A comparison of various analogues and partial structures of fenamic acids shows that the diphenylamine moiety is the minimal structure that can serve as a moderately potent competitive inhibitor of PG synthetase (Cushman and Cheung, 1976). The ortho-carboxyl group and alkyl substituents enhance this potency.

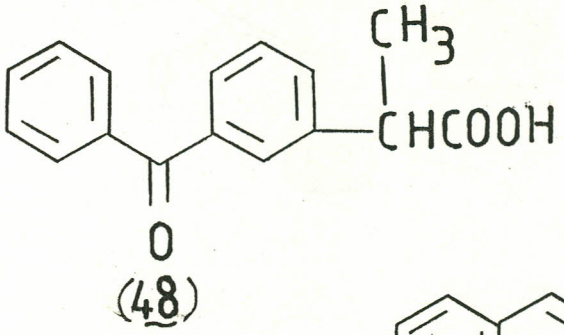
The importance of the carboxylic acid function in salicylates and other acidic NSAID is well recognised in their PG synthetase inhibitory activity. Several classes of


F
CH2COOH
CH3
CH
S→O
CH3

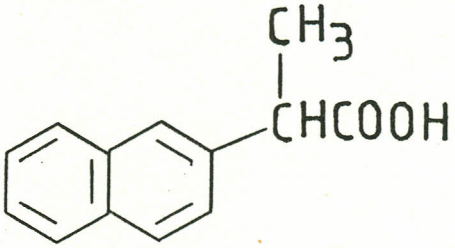
(46)


(CH3)2CHCH2
CH3
CHCOOH

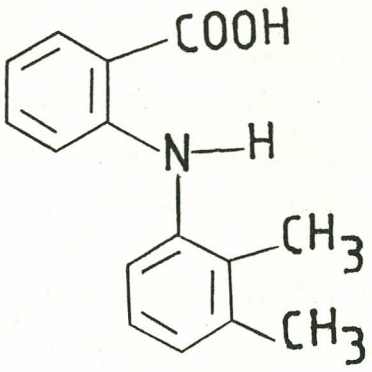
(47)


CH3
CHCOOH
O

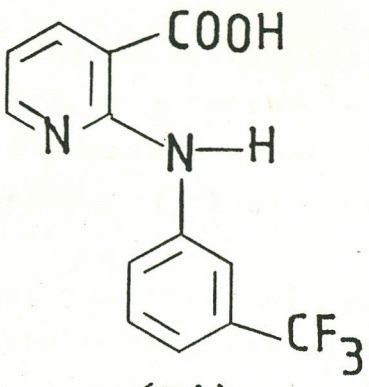
(48)


CH3
CHCOOH

(49)



(50)



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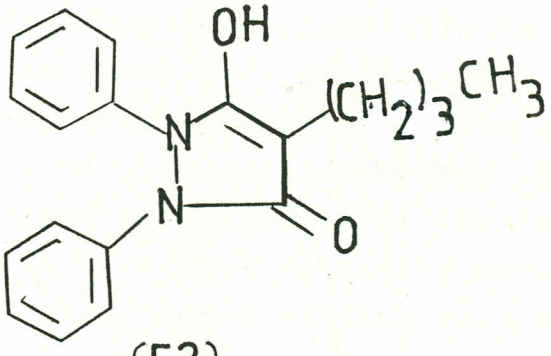

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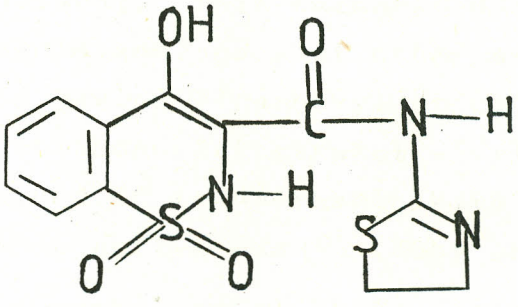
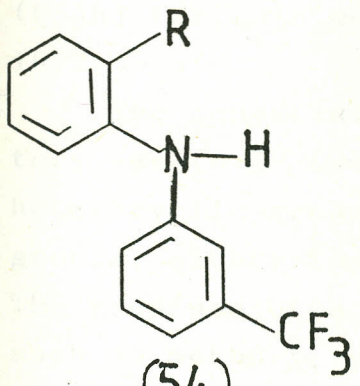


(53)

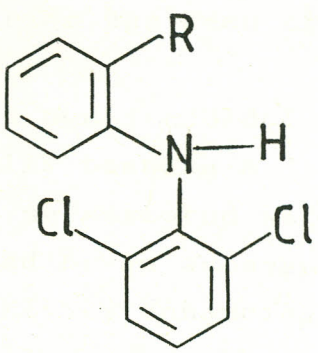
aromatic structures bearing an acidic function such as an enolic hydroxyl or a tetrazole group are also active anti-inflammatory agents. These are for example Phenylbutazone (52) and Sudoxicam (53).

The observation that the tetrazole group has an acidic hydrogen which compares with that of the carboxyl group in its pKa value(Mihina and Herbst,1950; McMannus and Herbst, 1952), has led a number of medicinal chemists to replace the carboxyl group in biologically active compounds with a tetrazol-5-yl group. Such replacement has given encouraging results in the area of NSAID. A series of 5-(2-anilinophenyl)- tetrazoles were synthesised as analogues of the fenamic acids, and these have shown activity comparable with that of their corresponding carboxylic acids. The most active anti-inflammatory tetrazoles in this series were (54) and (55) (Juby,Hudyma, and Brown.1968). This encouraged the preparation of tetrazole analogues of other anti-inflammatory agents having a carboxyl group. A series of 1-substituted 3-(tetrazol-5-ylmethyl)indoles as analogues of indomethacin have been synthesised. The most active was (56) (Juby and Hudyma,1969).

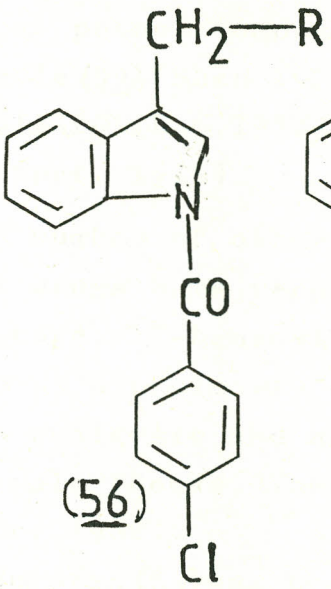
A number of aryltetrazolyl alkanoic acids have been prepared and maximum anti-inflammatory activity was found in those members of the series which have a meta-halogenated aromatic substituent and a propanoic acid residue at position 2 of the tetrazole ring (Buckler,Hayao,Lorezent, Sancilio, Hartzler and Strycker,1970), as well as the amides (Buckler, 1971), for example (57) and (58). The Quantitative Structure-Activity Relationships


R
N—H
CF3

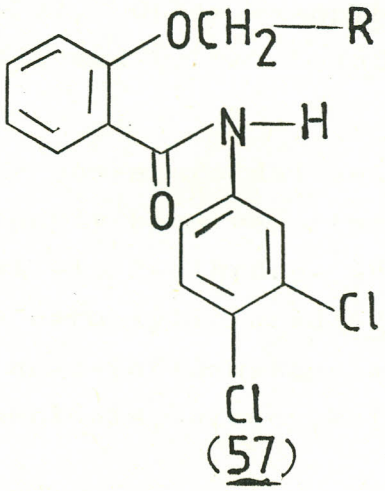
(54)


R
N—H
Cl
Cl

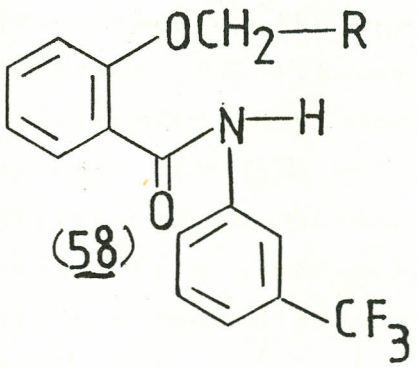
(55)


CH2—R
N
CO
Cl

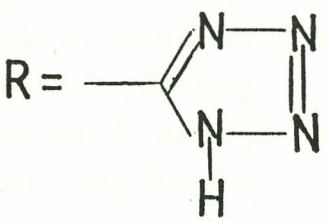
(56)


OCH2—R
N—H
O
Cl
Cl

(57)


OCH2—R
N—H
O
CF3

(58)

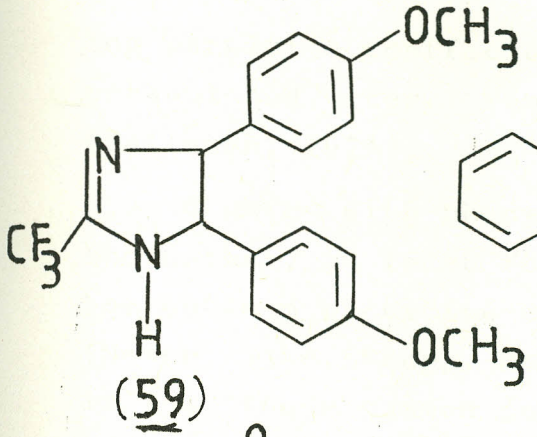

R=
N—N
N—N
H

(QSAR) for this series has been studied (Buckler, 1972).

The non-acidic non-steroidal anti-inflammatory agents generally possess a 5-membered heterocyclic group substituted with two or three phenyl groups, either fused or in an angular fashion. The presence of activity enhancing substituents such as methoxy, halogen or other hydrophobic groups is also a feature of these compounds. The most potent compound of this type is Flumizole (59) (Shen,1979). Other examples are Indoxole (60) (Ham,1972), and Diftalone (61)(Carminati and Lerner, 1975).

A number of other non-steroidal anti-inflammatory drugs have recently been reported in the literature. 5-benzoyl -1, 2-dihydro- 3H-pyrrolo (1, 2α -) pyrrole -1- carboxylic acid (62) shows potent analgesic and anti-inflammatory activity in animals (Rooks, Tomolonis,Maloney,Wallace and Schuler, 1982).
Benoxaprofen (63) has been reported to inhibit polymorphonuclear leucocyte migration as a consequence of its pro-oxidant properties (Anderson, Lukey,Nande and Joone, 1984).
Morniflumate, the morpholino - ethyl ester of niflumic acid (51), retains the anti-inflammatory activity of the acidic parent acid but has freedom from ulcerogenic effects of the acidic parent compound(Schiantarelli, Cadel, and Acerbi, 1984).

Physicochemical studies on acidic NSAID have been reported in the literature. These compounds have pKa values between 4 and 5. In addition, most of the compounds display a roughly similar degree of lipophilicity, as reflected in their



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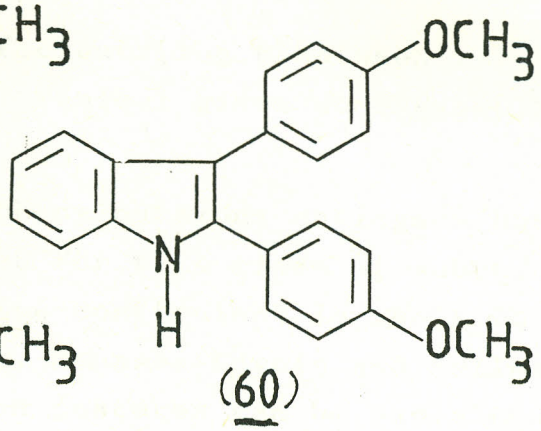
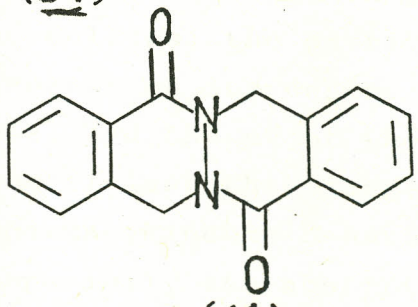
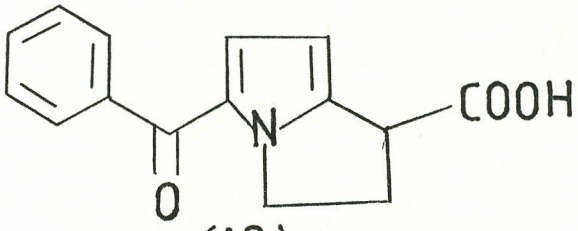


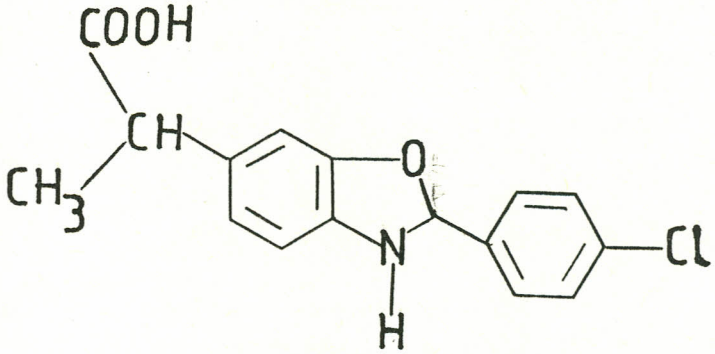
(60)



(61)



(62)



(63)

log partition coefficients(log P) between 1-octanol and water. Table 1 gives some examples (Sallmann, 1976).

Studies with phenylbutazone analogues have shown that, at least for this class of substances, the optimum partition coefficient is about 10 (Moser, Jake, Krupp, Menasse-Gdynia and Sallmann, 1975). These common features can be explained as follows: the partition coefficient of an acid depends on its degree of dissociation i.e. on its pKa, and the pH of the medium; it is this coefficient that largely determines the pharmacokinetic behaviour of any drug, including especially its absorption, its binding to plasma proteins and receptors and its excretion.

Table 1

pKa values and Log partition coefficients (Log P) between 1 - octanol and water for various acidic anti-inflammatory drugs

Drug	pKa	Log P
Phenylbutazone	4.8	0.701
Indomethacin	4.2	1.004
Diclofenac	4.0	1.127
Ibuprofen	4.5	1.104
Ketoprofen	4.0	0.000
Naproxen	4.5	0.342

(ii) Steroids

Progesterone, testosterone and aldosterone are all devoid of anti-inflammatory activity (Kappas and Palmer, 1963). Deoxycholic acid has been shown to cause the release of prostaglandins (PGs) in the gut (Beubler and Juan,1979). Grossman (1972) found that estrogen treatment increases the content of certain glycosaminoglycans (GAGs) and decreases collagen content. The estrogen-induced increase in either content or degree of polymerisation of GAGs has been postulated as a contributory factor in the vessel-protecting and acute anti-inflammatory effects of estrogens(Bonta and de Vos,1965). The vascular anti-inflammatory effects of topically administered estrogens was demonstrated in an uncoventional model of inflammation, which involved the induction of tissue damage by snake venoms(Bonta, Vargafting and Bohm, 1979) or their specific constituents (Bonta and Vargaftig,1976). In this model, antihistamines, NSAID, corticosteroids and PG antagonists and protease inhibitors proved ineffective. It thus appears that some kind of unusual anti-inflammatory effect can be exerted by the estrogens. Estrogens were also shown to stabilise the membranes of lysosomes and liposomes(Persellin and Perry,1972, Weissman and Rita, 1972). Inhibition of the oxygen consumption of phagocytozing leucocytes and during PG biosynthesis have also been described for estrogens(Bodel,Gillard,Kaplan and Malawista, 1972; Lerner,Carminati and Scliati,1975). It is conceivable that some or all of these mechanisms account for the estrogen-induced inhibition of adjuvant arthritis in rats and also for the benefit which estrogens provide in some cases of clinical rheumatoid arthritis(Glenn,1966;

Toivanen, Maatta, Suolanen and Tykklainen,1967; Spangler, Antoniades and Sotman, 1969). It has also been proposed that estrogens at least contribute to the beneficial influence of pregnancy on arthritis (Bonta,1979).

Whatever the mechanism may be, there are several observations on the basis of which re-evaluation of estrogens as putative anti-rheumatic agents seems to be warranted. The potent hormonal activity is obviously a major drawback to the clinical anti-arthritic use of estrogens in a male population. However, there are some indications that the hormonal and anti-inflammatory properties of estrogens may be dissociated from each other. For example, the vessel protecting activity of estrogens is also displayed by steroids exhibiting the chemical structure of estrogens but devoid of estrogenic endocrine properties(Bonta,1979). These steroids have not been evaluated however, in other inflammatory models. Thus the putative development of such estrogens for use in arthritic disorders remains an untested possibility as yet.

Since the discovery that cortisone was effective in the treatment of rheumatoid arthritis (Hench, Kendall, Slocumb and Polley,1949), intense efforts have been made toward modifying its structure in the hope of finding related compounds with superior properties. When corticosteroids are used in doses necessary to suppress symptoms of rheumatoid arthritis, they also affect other metabolic processes. Side

effects such as excessive sodium retention and potassium excretion, negative nitrogen balance and potassium excretion

increased gastric acidity, oedema and psychoses, are exaggerated manifestations of the normal metabolic functions of these hormones.

It was recognised quite early that a carbonyl group at C-3, a Δ^4- double bond and an oxygen or β- OH at C-11, as well as a β-ketol side chain at C-17, are essential for gluco-corticoid activity. Analogues containing these functions were therefore synthesised. Noteworthy candidates have included $\Delta^{1,4}$-analogues of cortisone and hydrocortisone, namely prednisone and prednisolone respectively (Herzhog,Nobile, Tolksdorf,Charney, Hershberg and Perlmann,1955). These compounds are more potent anti-rheumatic agents than the parent compounds and produce fewer undesirable side effects. The increased potency may reflect the change in geometry of rings A caused by the introduction of the C = C function. The conformation of this ring changes from a half -chair in Δ^4-3-ketosteroids, to a flattened boat in $\Delta^{1,4}$-3-ketosteroids.

A 6 α -methyl analogue was introduced since it was thought that this would slow the metabolism of the compound by slowing the reduction of ring A (Hogg, Spero, Thompson, Lincoln and Schneider, 1957). Such compounds showed increased glucocorticoid activity with less salt retention.

The 9 α -bromo analogue of 11 β-hydroxy cortisol was synthesised (Fried and Sarbo,1953) and had $^{1}/_{3}$ the activity of cortisone acetate. Other halogens were introduced into the 9 α - position and it was soon observed that glucocorticoid activity was inversely proportional to the size of the halogen at C-9. The 9 α - fluoro analogue of cortisone is eleven times as potent as cortisone acetate. Fludrocortisone has three hundred to eight hundred times the activity of cortisone in its mineralocorticoid activity (Robinson,1959;Stuart,1957).
Triamcinolone combines a 9 α -fluoro with $\Delta^{1,4}$- double bonds. The mineralocorticoid potency conferred by the 9 α -fluoro is to some extent attenuated in this compound by a 16 α -hydroxyl group. The original interest in 16 α -hydroxy corticosteroids stemmed from their isolation from the urine of a patient with adrenal tumour. Analogues were synthesised in the hope that these may have potent biological activity (Hirshman,Hirshman and Farrel,1953).

Research with 16-methyl substituted corticoids was initiated in part because investigators hoped to stabilise the 17β -ketol side chain to metabolism in vivo. A 16- methyl group does decrease the reactivity of the 20-keto group

to carbonyl reagents and increases the stability of the drug in human plasma in vitro (Arth,Fried, Johnston,Hoff, Sarret, Rilbur,Stoerk and Winter 1958;Oliverto,Rausser,Nussbaum,Gebert and Hershberg,1958). Unlike 16-hydroxylation, the methyl group appears to markedly reduce the salt-retaining properties of the compound. These studies led to the development of dexamethasone (Sperber, 1962;Welsh and Ede,1962). A 6 α -fluoro group does not deleteriously affect the activity of prednisolone hence its 6 α -fluoro derivative (Boland,1962).

Unlike the acidic non-steroidal anti-inflammatory agents, corticosteroids show a general increase in activity as log P(1-octanol) value increases (Table 2).

Table 2

log P (1-octanol) values and anti-inflammatory activity for various corticosteroids

Drug	+log P(1-octanol)	*Anti-inflammatory activity
Hydrocortisone	0.89	1.0
Prednisolone	1.42	4.0
Prednisone	1.46	4.0
Fludrocortisone	1.68	6.0
Methylprednisolone	1.85	6.0
Betamethasone	1.98	70
Dexamethasone	1.90	200

+ Hansch, Leo and Elkins, 1971

* Bowman, Rand and West, 1968