REVIEW ARTICLE

BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS.

PART I. SYNTHETIC NITROGENOUS STEROIDS

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THE broad spectrum of biological activity found within the group and the multiplicity of actions displayed by certain individual members, make the steroids one of the most intriguing classes of biologically-active compounds. Structural modification studies, whose extent is unequalled in any other area of medicinal chemistry, have not only furnished so many steroidal derivatives that structure-action relationship studies are possible to a degree undreamt of in other fields, but they have also led to the introduction of several cheaper, safer, more specific and more potent therapeutic agents. Among the many steroidal derivatives now known, are a number of compounds which incorporate nitrogen atoms in their molecular structure, and it is the purpose of the present review to indicate the importance of these nitrogenous steroids and to evaluate, where possible, the influence of the nitrogen atoms upon the biological activity displayed. Certain aspects of the subject have been reviewed previously, particularly the pharmacology of the veratrum alkaloids—for more recent reviews see Abreu (1959), Hoobler and Dontas (1953), Krayer (1958), Stoll (1954). The earlier work is exhaustively reviewed by Krayer and Acheson (1946) who refer to previous review articles. A brief survey of the biological properties of nitrogenous steroids in general has also been published (Voigt and Kallistratos, 1957).

To set the field in perspective the present review will consider nitrogenous steroids in several contexts. Suitable examples, chosen mainly from the synthetic derivatives, will be used to illustrate how the group fits in with modern concepts of drug action and a brief survey will then be given of the biological properties of the rapidly expanding group of known steroidal alkaloids. With the large number of nitrogenous steroids now known, it is quite impossible to achieve a complete coverage of the pertinent literature, but a serious attempt has been made to make the survey as representative as possible.

Clinical Implications

At the present day clinical application of steroids possessing nitrogen atoms is very limited, although there are several indications that the full potentialities have not yet been realised. This is particularly true in the field of synthetic derivatives, as it is only within the past few years that

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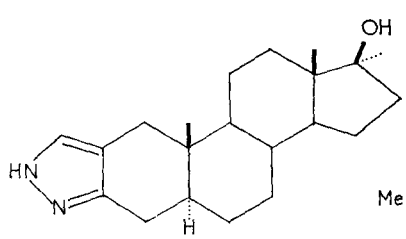
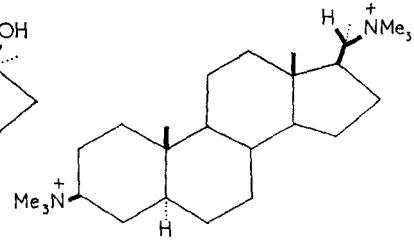
the intensive search for modified steroidal hormones, showing high specificity or accentuating a minor or secondary biological characteristic of the natural analogue, has been extended to include more than a handful of nitrogenous derivatives. It does not therefore seem unreasonable to anticipate that among new nitrogenous steroids will be found compounds exhibiting clinically desirable carcinolytic properties, improved anabolic to androgenic ratios or superior lipodiatic to oestrogenic indices. Indeed certain steroidal [3, 2-c]-pyrazoles (Clinton and others, 1959, 1961b) and [2, 3-d]-isoxazoles (Clinton and others, 1961a) are already known to possess very favourable anabolic to androgenic ratios. Moreover, as these derivatives are active by the oral route and one representative, 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I), has shown



I

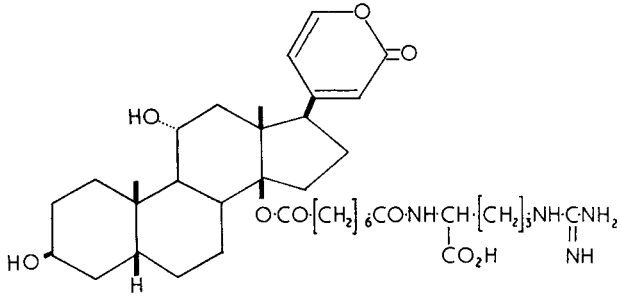


II

promise on preliminary clinical trial (Howard and others, 1959), such compounds may well find a permanent place as therapeutic agents. Anabolic properties are also present in other nitrogenous steroids including 17β-hydroxy-17α,2′-dimethylandrostano-[3,2-b]-thiazole, certain *N*-substituted 2-aminomethylene-17α-methyldihydrotestosterones (Zderic and others, 1960) and various substituted 16α-aminopregnenes (Rhone-Poulenc, 1960).

The possibility of securing new therapeutic agents amongst nitrogenous steroids is by no means confined to synthetic derivatives, however, and naturally-occurring compounds may also have their role to play. Thus the recently characterised bisquaternary steroidal alkaloid, malouetine (II), which occurs in *Malouetia bequaertiana* (Janot, Lainé and Goutarel, 1960), has been shown in preliminary experiments to possess competitive neuromuscular-blocking potency equal to that of (+)-tubocurarine whilst being only one third as toxic (Quévauviller and Lainé, 1960) and so this compound or related drugs could conceivably offer alternatives to tubocurarine as an adjunct to surgery. The three possible isomers of malouetine involving the configurations of the nitrogen atoms, namely the diquaternary bases in which the nitrogen atoms are in the 3β-20β-, 3α-20α- and 3α-20β- configurations have been prepared synthetically (Goutarel, 1961) and it will be of some interest to learn of their relative potencies. Again, the steroidal alkaloid funtumidine, on the basis of motility tests in rats, has been claimed to possess tranquillizing properties comparable to those of reserpine (La Barre and Desmarez, 1959) and so alkaloids of this type may have a role to play in this area of clinical medicine.

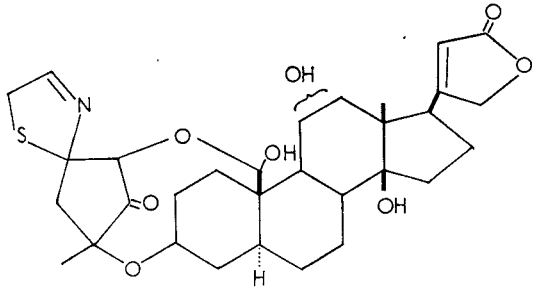
Certain clinically acceptable nitrogenous steroids are found in nature but are not used because cheaper or superior agents are available. Examples are the suberylarginine derivatives of the bufadienolides, such as bufotoxin and gamabufotoxin (III), which are constituents of the poison



III

secreted by the parotid glands of the toad, and which have similar activity to the plant cardenolide and scilladienolide glycosides employed clinically in the treatment of congestive heart failure. It is of some interest that the toxic principles secreted by the salamander are also nitrogenous steroid derivatives (Schöpf, 1961) although these compounds are of a different chemical type and are without potential clinical application.

Nitrogenous derivatives of cardenolides are also found in plants. Examples are uscharine (IV) (Hesse and Mix, 1959) and its dihydroderivative voruscharine (Hesse and Ludwig, 1960) which occur in the latex



IV

of *Calotropis procera.* These compounds do not appear to have seen clinical trial but uscharine has been shown to have a potency 58 per cent of that of ouabain in the etherised cat (Chen, Bliss and Robbins, 1942).

The nitrogenous steroids which have seen the most extensive clinical application are the steroidal alkaloids of the protoveratrine type which produce a reflex fall in blood pressure through a generalised vasodilatation and fall in heart rate. Crude and complex mixtures of these alkaloids saw a certain amount of clinical use around the turn of the century, but their toxicity and unpleasant side effects brought them into disfavour. The use of the purified principles saw a resurgence of popularity several

years ago with the heightened interest in the problem of hypertension (see for example Meilman and Krayer, 1952; Meilman, 1959; Robson and Keele, 1956), especially as they produce vasodilatation in all peripheral circuits including the brain and kidneys, and are free from the disturbance of the postural reflexes produced by ganglion-blocking agents. Unfortunately these highly desirable physiological properties are more than offset by the narrow margin between the therapeutic and toxic doses and the fact that emesis nearly always occurs with therapeutic doses. Hence these alkaloids have been virtually eliminated from clinical use, although they may still find occasional application in the treatment of certain toxaemias of pregnancy (see for example Finnerty and Fuchs, 1953; Meilman, 1953; Krupp and others, 1956).

The fact that the protoveratrine group of alkaloids afford such a clinically desirable integrated response, and yet cannot be employed because of their side effects, has presented a most tantalising challenge to the medicinal chemist and numerous attempts have been made to prepare synthetic nitrogenous steroids retaining the hypotensive properties, but devoid of the side effects. So far these efforts have met with little success. Among the compounds prepared are numbered several cholylamine esters (Fieser and Wei-Yuan Huang, 1953), certain ternorcholanylthiazoles (Dodson, 1955a), various 16α-aminopregnenolones (Rhone-Poulenc, 1960; Gould and others, 1956), some aminoalkanol esters of 17α-hydroxy-3-ketoandrost-5-en-17β-carboxylic acid (Bloom, 1956) and two 17-imidazolylandrostenes (Sturtevant, 1958).

Other steroidal alkaloids have seen limited clinical trial in the treatment of conditions other than hypertension. For example the "Kurchi" alkaloids from various *Holarrhena* spp. have been employed in the treatment of amoebic dysentery, both free and in the form of bismuth iodide complexes (see for example Acton and Chopra, 1933; Tanguy, Robin and Raoult, 1948; Lavier, Crosnier and Merle, 1948) and *Solanum* alkaloids were once used in the treatment of asthma and neuralgia (Leclerc, 1938), but neither class is of any great value.

SYNTHETIC NITROGENOUS STEROIDS AND THEIR PLACE IN MODERN THEORY

The lack of knowledge of the principles by which biological activity is related to chemical structure has necessitated numerous tedious structural modification studies in which the medicinal chemist has sought to improve upon known drugs of proven efficacy, and many steroids possessing nitrogen atoms have played their role in this work. Usually the preparation of such compounds has been conducted on purely empirical grounds but occasionally it has followed from the application of theoretical concepts such as the receptor theory of drug action, the theory of metabolite displacement, the concept of bioisosterism or the supporting moiety theory. In the following sections examples will be given of nitrogenous steroids which have either been prepared from consideration of these concepts or which can be discussed in retrospect in terms of these concepts. It is felt that this treatment will place the compounds in their correct perspective and at the same time afford a comprehensive survey without giving rise

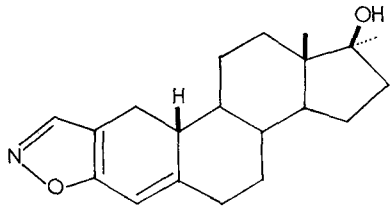
merely to a catalogue of the various synthetic nitrogenous steroids which have been studied biologically.

Receptor Theory

Although very little is known of the intimate nature of the hypothetical "drug receptors" in the tissues, the receptor theory has proved an extremely useful aid to the medicinal chemist in his attempts to rationalise drug action in so far as it stresses the importance of the 3-dimensional geometrical shape and electronic distribution of the drug molecule. It was consideration of the receptor theory which led to the planned synthesis of the anabolic steroidal [3,2-c]-pyrazoles and [2,3-d]-isoxazoles, where it was assumed that the receptors concerned with the androgenic and anabolic properties of the natural male hormones differed in their nature (Clinton and others, 1961b). Attention was concentrated on securing an alteration of the intergroup distance between the substituents at C(3) and C(17), with an accompanying change in the nucleophilicity of the C(3) substituent, and satisfaction of these requirements led logically to the preparation of the pyrazole and isoxazole derivatives.

After oral administration 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole (I) proved to be some 30 times as potent as 17α-methyltestosterone in the rat nitrogen retention test, whilst it was only one quarter as androgenic in the ventral prostate weight gain test (Arnold, Beyler and Potts, 1959). In the levator ani muscle test in immature castrated male rats it proved to be twice as myotrophic when given by the oral route as 17α-methyltestosterone (Potts, Beyler and Burnham, 1960), and it also proved effective in reversing the catabolic actions of cortisone acetate in the same animals (Beyler, Potts and Burnham, 1960). Surprisingly, acylation of the pyrazole ring imparted some oestrogenicity to the pyrazole series and a 6α-methyl group decreased both the androgenic and anabolic properties (Clinton and others, 1961b).

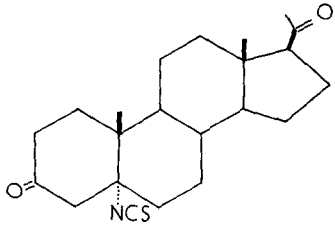
The isoxazole derivatives show broadly similar activity to the pyrazole compounds (Clinton and others, 1961a; Zderic and others, 1960) although one, 17β-hydroxy-17α-methyl-19-norandrost-4-eno-[2,3-d]-isoxazole (V), showed a conspicuous lack of specificity. Thus it exhibited progestational



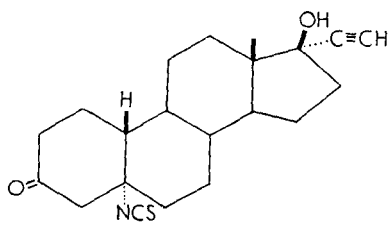
V

activity equal to progesterone on intramuscular administration and to ethisterone on oral administration, as well as showing anabolic, myotrophic, androgenic and oestrogenic properties (Clinton and others, 1961a).

In their visualisation of drug-receptor interaction Van Rossum and Ariens (1957) suggest that the "drug-receptor complex" is basically an interaction of fields of force originating in the drug molecule and in the tissue. Electrostatic and van der Waals forces play the dominant role, and it is postulated that certain specific interactions within the general field determine the intrinsic activity or ability of the drug to evoke the biological response. If this representation is correct then maintenance of the general interaction with concurrent variation in electron density at certain specific areas might be expected to produce large changes in intrinsic activity without appreciable changes in the affinity for the receptor. In the glucocorticoid field such a situation would appear to arise from the introduction of an electron-withdrawing substituent in the form of a 9α-fluorine atom which greatly enhances potency. The spectacular success of this introduction of a 9α-fluorine atom has logically led to investigations of the effect of introducing other electron-withdrawing groups at various positions in the steroid nucleus and among the compounds so prepared are several with groups containing nitrogen. Thus several 5α-, 7α-, 9α- and 11β-thiocyanato-steroid hormone derivatives have been synthesised (Kawasaki and Mosettig, 1959; Schaub and Weiss, 1961; Takeda, Kubota and Kawanami, 1960) and it was discovered that 3,20-dioxo-5α-thiocyanatopregnane (VI) and 17α-ethynyl-17β-hydroxy-3-oxo-5α-thiocyanato-19-norandrostane (VII) were approximately equal in



VI



VII

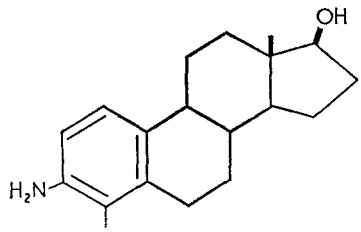
progestational activity to their parent compounds progesterone and 19-norethynyltestosterone respectively. Similarly the 4,5-dihydro-5α-thiocyanato-analogue of cortisone acetate showed comparable activity to cortisone acetate (Takeda, Kubota and Kawanami, 1960) although the 4,5-dihydro-5α-thiocyanato-derivative of hydrocortisone acetate showed little or no activity. Doubt is expressed, however, whether the thiocyanato-derivatives were themselves active in view of their ready reconversion into the parent hormone.

Other steroid hormone analogues with electron-withdrawing groups in the molecule include various 5- and 6-cyano-derivatives (Bowers, 1961; Bowers and others, 1959) and several 6-nitro compounds (Bowers, Ibáñez and Ringold, 1959; Bowers, Sánchez and Ringold, 1959). Of these, 6α-nitro-17α-acetoxyprogesterone was found in the Clauberg assay, oral route, to be 3–4 times as active as 17α-acetoxyprogesterone as a progestational agent (Bowers, Ibáñez and Ringold, 1959). On the other

hand both 6α- and 6β-nitrotestosterone were inactive as myotrophic, androgenic or gonadotrophin-suppressing agents in the parabiotic rat (Bowers, Sánchez and Ringold, 1959). The isomeric 6α- and 6β-nitroprogesterones exhibited less than one eighth the progestational activity of progesterone in the guinea-pig copulatory assay (Bowers, Sánchez and Ringold, 1959) and 21-nitroprogesterone was inactive (Bowers and Ringold, 1959).

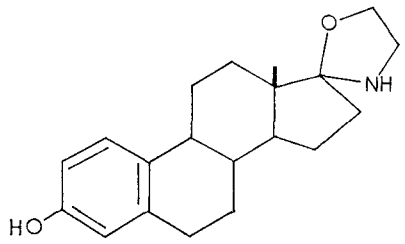
The 2-nitro-, 4-nitro- and 2,4-dinitro-derivatives of oestrone (Werbin and Holoway, 1956) and oestradiol (Patton, 1959b) are known, but like the 16-isonitroso-derivative of oestrone-3-methyl ether (Litvan and Robinson, 1938) they do not appear to have been tested biologically.

Various nitrogenous steroids, where the nitrogen forms part of an electron-donating group, have also been studied. In particular considerable attention has been paid to derivatives of oestrone and oestradiol. Amongst such compounds may be mentioned 2-amino-4-methyloestra-1,3,5(10)-trien-17β-ol (Dannenberg and others, 1960), 3-amino-4-methyl-oestra-1,3,5(10)-trien-17β-ol (VIII) (Dannenberg and others, 1959) and



VIII

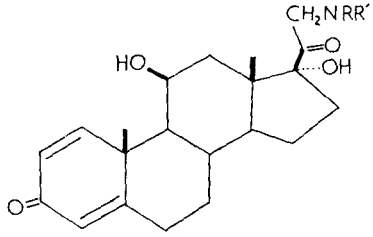
certain 2-dialkylaminomethyl derivatives (Patton, 1959a; 1960) which all proved devoid of activity. It is claimed however that a derivative of oestrone thought to possess the 17-spiro-oxazolidine structure IX exhibits an oral activity ten times that of oestrone (Hebo, 1951).



IX

Several synthetic steroids possessing the provitamin D 5,7-diene system with a terminal tertiary amino-function in the side chain have been prepared but on irradiation, only slight anti-rachitic activity was observable in the most favourable substance (Louw, Strating and Backer, 1955). The amides from which these amines were prepared were also inactive on irradiation.

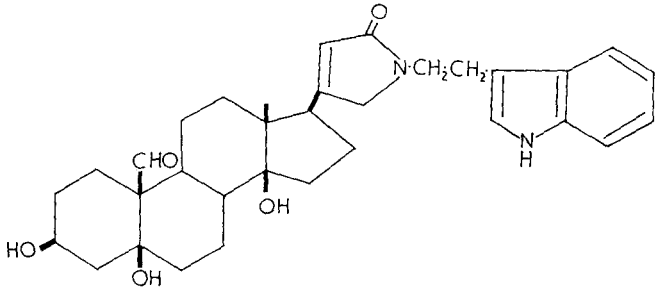
Other examples of the introduction of an electron-donating nitrogen function into the steroid side chain are afforded by a number of *N*-substituted 21-amino-11β,17α-dihydroxypregna-1,4-diene-3,20-dione compounds of type X, which have been shown to retain the glucocorticoid



X

activity of prednisolone from which they are derived, as evidenced by the results of liver glycogen accumulation and rat foot oedema tests (Tóth, Tuba and Szporny, 1961).

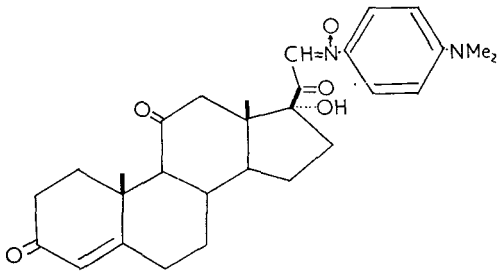
More complex structural modification studies involving nitrogen have been reported in the cardenolide field. Thus strophanthidin reacts with primary and secondary amines to form nitrogenous derivatives (Bembry, Elderfield and Krueger, 1960), one of which, tryptamine-strophanthidin, not only retains a typical digitalis-like action on the isolated papillary muscle of the cat (Greiner and Reilly, 1952), but unlike the glycosides of the strophanthus series, it is active by the oral route in man (Otto and others, 1953). Unfortunately, however, it often produces emesis. The structure originally assigned to this compound (Otto and others, 1953) has since been retracted (Bembry and others, 1960) and it is now believed to be that shown in XI. Other cardenolide derivatives which have been



XI

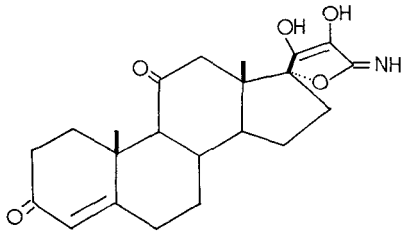
studied biologically include the 3-diethylaminoacetate and the 3-nicotinate of strophanthidin (Küssner, 1939; Steldt, Anderson and Chen, 1944). In the cat the diethylaminoacetate proved more potent than the parent aglycone, but the nicotinate was less active (Steldt and others, 1944).

Two *p*-dimethylaminophenylnitrones related to cortisone and hydrocortisone, viz. 17α-hydroxy-3,11,20-trioxopregn-4-en-21-*p*-dimethylaminophenylnitrone (XII) and the corresponding 11β-hydroxy compound,



XII

have been shown to retain glucocorticoid activity as demonstrated in the liver glycogen deposition assay (Leanza and others, 1954), but several 21-pyridinium salts derived from cortisone and hydrocortisone were inactive, as was the iminolactone XIII.



XIII

There are a number of references in the literature to the screening of various nitrogenous steroids only remotely related structurally to steroid hormones, and as might be expected, in most tests these compounds proved inactive, as, for example, the series of 24-amino-derivatives prepared from bile acids which showed no antirheumatic activity (Wessely and Swoboda, 1951). It is therefore a little surprising that certain substituted amino-alkanol esters of bile acids appear to exhibit some anti-inflammatory activity based on claims of their efficacy in the treatment of fibrositis and certain types of arthritis (Burtner, 1951).

Considerable attention has been devoted to structural modifications of the steroid nucleus itself and a number of these studies have been concerned with the introduction of nitrogen atoms into various ring positions. At the present time, in addition to several homoaza-steroids, aza-steroids are known in which each secondary carbon atom of the steroid nucleus, with the exceptions of C(1) and C(11), has been replaced by nitrogen (see for example Doorenbos and Mu Tsu Wu, 1961; Gut and Uskokovic, 1961; Knof, 1961; Kutney and Johnston, 1961; Jacobs and Brownfield, 1960; Shoppee and Krueger, 1961). Little work appears to have been published on the biological properties of these compounds so far, but they can be expected to provide interesting information in terms of the receptor theory with their regions of high electron density actually incorporated in the nucleus. Certain oxygenated 12a-aza-C-homo-steroids

have been reported (Mazur, 1957a,b) to inhibit the harmful deposition of liver glycogen occurring as an untoward effect in cortisone therapy and 4-aza-pregn-5-en-3,20-dione is claimed to exhibit marked anti-inflammatory activity in rats (Wildi, 1959). Weak androgenic properties and anti-oestrogenic activity are present in certain lactams belonging to the 4-aza-androstane series (Doorenbos and Huang, 1961). Several bis-dehydrodoisynolic acid analogues possessing the 1,2,3,4-tetrahydro-benz-[f]-isoquinoline nucleus were inactive as oestrogens and also failed to exhibit any androgenic or anti-inflammatory activity (Nelson and Hsi, 1961).

Nitrogenous Steroids as Antimetabolites

Where the affinity for a receptor fitted by a normal metabolite of a living organism is also present in a synthetic analogue of the metabolite, but the analogue exhibits a greatly reduced intrinsic activity, then the analogue is likely to function as an antagonist of the metabolite. Recently there has been an intense interest in the planned synthesis of antimetabolites, and this has been referred to as "the revolution in pharmacology" (Woolley, 1960). In the steroid field, several antimetabolites, such as the spirolactone antagonists of aldosterone (Atwater and others, 1961; Barter, 1960) are well established whilst other compounds, such as the halogenated analogues of the corticoid steroids (Fried, 1957) and the dihydrocardenolides (Cosmides, Muja and Carr, 1956) are believed to competitively antagonise their parent compounds. Yet nitrogen-containing steroids seem to have been little investigated as metabolite-displacing agents. Cholesterylamine, however, has been demonstrated to be a weak inhibitor of the use of cholesterol by the cockroach (Noland, 1954) and there are claims in the patent literature (Dodson, 1955b; Rorig, 1953) that certain nitrogenous steroids possess antihormonal activity. It is also possible that the antimicrobial activity displayed by various nitrogenous steroids may be due to antagonism towards steroidal metabolites of the organism, although it is clear that many of these compounds act by virtue of their surface-active properties (Stacey and Webb, 1947a). Such surface activity is widespread within the steroid field, and is particularly pronounced in the saponins, cardiac glycosides, various steroidal alkaloids, and the bile acids. In addition to conferring antimicrobial properties, the surface activity also confers haemolytic properties and it is therefore not unexpected that 3,6-diaminocholestane is a haemolytic agent (Stückradt, 1939).

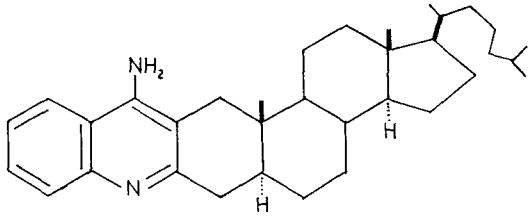
A large number of nitrogenous steroids have been tested for antibacterial and antifungal activity. These compounds cover a wide range of chemical complexity and include both synthetic compounds and alkaloids. Among the simpler synthetic compounds may be mentioned the epimeric 7-amino-cholesterols (Barnett, Ryman and Smith, 1946a), various mono- and di-aminocholestane derivatives with the nitrogen functions in the 3-, 6- or 7-positions (Barnett, Ryman and Smith, 1946b) and several hydroxylated 23-aminonorcholane derivatives, including 23-guanido-3,7,12- trihydroxy-norcholane (James and others, 1946). The monoaminocholestane

derivatives showed some activity against Gram-positive organisms (Barnett and others, 1946a,b) and the potency is increased in the diamino-compounds which in addition, showed some activity against Gram-negative organisms (Barnett and others, 1946b).

Amino-steroids prepared from bile acids in which the nitrogen atom is attached directly to the steroid nucleus exhibit but weak antibacterial properties (Hilton, Jones and Westwood, 1955; Jones, Webb and Smith, 1949; Redel and others, 1951) but where the amino-group is in the side chain, the potency is higher (Hilton and Webb, 1951; Stacey and Webb, 1947b).

Among the more complex synthetic nitrogenous steroids which have been shown to exhibit antibacterial properties are a number of *N*-substituted 16-amino-derivatives (Schering, 1955) and 3,3-di(*N*-acetyl-*p*-aminophenylmercapto)-7,12-diketocholanic acid (Jones, Smith and Webb, 1948). Several *N*-substituted 22-aminobisnorcholanes and their derived quaternary methiodides were found to possess antifungal activity in tests with *Candida albicans* (Herzog, Payne and Hershberg, 1955), but the amides from which they were derived showed little or no activity. Various aminopregnane derivatives of varying chemical complexity also possess antimicrobial activity (Kull, Castellano and Mayer, 1953; Micheli and Bradsher, 1955). The anti-amoebal properties of the steroidal alkaloid conessine inspired the screening of several synthetic nitrogenous steroids (Dodgson and Haworth, 1952), but none was more active than conessine itself. Certain compounds of this type showed antibacterial activity of about one twentieth that of streptomycin (Joska, Černý and Šorm, 1954).

Of a series of quinolino-, indolo-, pyrrolo-, thiazolo- and triazafluoreno-steroids prepared as potential antimicrobial agents only one, XIV, was



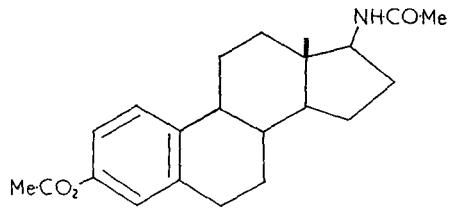
XIV

sufficiently soluble to be tested and it proved inactive (Antaki and Petrow, 1951). *N*-Phenyl-3β-cholestanamine and *N*-*p*-tolyl-3β-cholestanamine, which were prepared during a search for drugs effective in leprosy and tuberculosis, also proved to be inactive (Buu-Hoi and Cagniant, 1944).

Bioisosterism

Certain nitrogenous steroidal derivatives are of particular interest in terms of the concept of bioisosterism (Schatz, 1960; Friedman, 1951) which postulates that compounds in isosteric relationship should possess either similar or opposite biological activity. This behaviour can be rationalised in terms of the receptor theory of drug action since the great

similarity in chemical and physical properties shown by isosteres should ensure similar affinities for the same receptors, mimicry or antagonism then being determined by the intrinsic acitivities of the individual isosteres. In the extended definition of isosterism (Erlenmeyer, 1948), functional groups which are related by the Hydride Displacement Law of Grimm (1934 and earlier refs.) are considered to be isosteric and so such a relationship will pertain for steroids related by the substitution of the -NH-group for the -O- function. Unfortunately, however, this particular isosteric relationship is complicated by the greater willingness of the nitrogen atom to enter salt formation and so strictly comparable biological activities are not necessarily to be expected. At physiological pH the amino-steroids will be ionised and so may have difficulty in penetrating permeability barriers (Brodie and Hogben, 1957) and may not reach the receptor. Although most of the amino-isosteres of steroidal hormones which have so far been tested are inactive and lack the ability to antagonise their natural analogues, potent oestrogenic activity is present in 17β-acetamido-3-acetoxyoestra-1,3,5(10)-triene (XV) (Dannenberg, Scheurlen

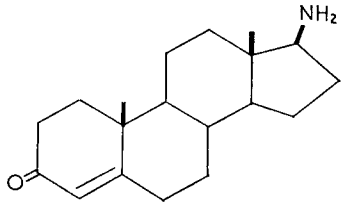


XV

and Simmer-Rühle, 1956) in which salt formation at the nitrogen atom is prevented by the amide function. The activity of this compound is also of interest in connection with the suggestion that oestrogenic activity is associated with the presence in the molecule of two groups capable of entering into hydrogen-bonding and which are held in a certain steric relationship (see for example Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). It has been further suggested that the distance between the two groups is a multiple of the "identity distance" (Long and Schueler, 1954) which is the distance between peptide links in a polypeptide chain.

The inactive oestrogen isosteres which have been reported are the 3-amino-isostere of equilenin (Bachmann and Dreiding, 1950) the 3-amino-, 17β-amino- and the 3,17β-diamino-isosteres of oestradiol and the 3-amino-isostere of oestrone (Hecker and Walk, 1960; Gold and Schwenk, 1959).

In the androgen series 3,17β-diaminoandrost-4-ene, 3-amino-17β-hydroxyandrost-4-ene and 3-oxo-17β-aminoandrost-4-ene have been reported to be devoid of male hormone activity (Joska and Šorm, 1956), but the analogue of testosterone, 17β-amino-3-oxo-androst-4-ene (XVI), although showing no activity in male rats, produced a pronounced increase in kidney weight and lesser increases in the liver and adrenal weights of female rats (Gaunt and others, 1954).



XVI

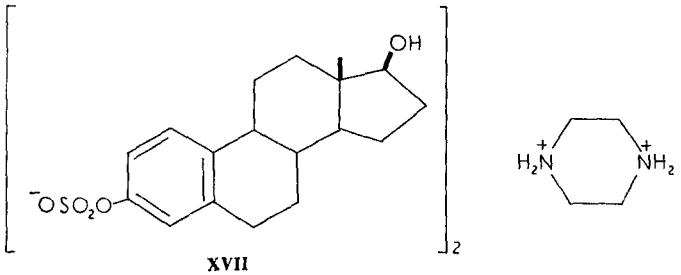
Replacement of the oxygen atom in the lactone ring of scillaren A by the-NH-group gave rise to a very sparingly soluble isostere which showed no cardiotonic activity when tested on the isolated guinea-pig auricle at a concentration of 10^{-6}M (Uhle and Schröter, 1961).

Drug Latentiation

Nitrogenous steroids have played a small but nevertheless significant role in drug latentiation (Harper, 1959) where a chemical derivative of an active drug is administered to overcome unfavourable rates of biotransformation or unfavourable solubility, distribution, transport and absorption characteristics—the active drug being regenerated *in vivo*. The steroidal moiety has sometimes functioned as the latentiating agent and at other times an active steroid has been latentiated.

Examples of the use of steroids as latentiating agents include the preparation of insoluble steroidal amine salts of penicillin capable of maintaining prolonged therapeutic concentrations of the antibiotic in the bloodstream (Coghill, Weston and MacCorquodale, 1950; Madinaveitia, 1955; Vaidya and Boyce, 1959) and the application of the cholesterol-6-sulphonate anion to yield an insoluble thiamine salt with which to enrich cereal flour (Mima, 1955). Another example is afforded by the choline salts of cholic acid and desoxycholic acid which exert actions typical of both moieties on the guinea-pig intestine (Meyer and McEwen, 1948). Attempted latentiation of 3-indolylacetic acid by the formation of steroid esters failed, however, to enhance parthenocarpic fruit induction in the tomato (Hofert and Sell, 1960).

Examples of nitrogenous moieties being used to latentiate biologically-active steroids are far more numerous. For instance much attention has been devoted to the preparation of amine salts of steroid hormone sulphate esters to increase the water solubility of the parent hormone. Among such compounds can be listed the ethylenediamine salts of the sulphate esters of oestrone, oestradiol, equilenin, androsterone and pregnenolone (Abbot, 1954a,b); the piperazine salts of the sulphate esters of oestradiol and equilenin (Hasbrouck, 1953); and the procaine (Deans and Scarrow, 1951), amphetamine (Grant, Glen and Barker, 1950) and 2-aminopyridine (Beall and Grant, 1952) salts of the sulphate esters of various steroidal oestrogens. Of these compounds piperazine oestradiol sulphate (XVII) has been used clinically. Dehydroandrosterone and androstenediol lose their biological activity if administered as dialkylamino sulphuric ester derivatives (Goisis and Polvani, 1955). Various quaternary ammonium

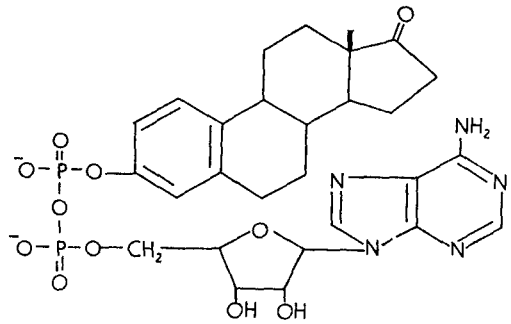


XVII

derivatives of hydrocortisone, prednisolone and dexamethasone retain activity (Mori and Nakagawa, 1961).

Latentiation of steroids may also be a natural phenomenon since amino-acid conjugates of steroidal hormones have been discovered in aqueous adrenal cortical extracts (Voigt and Schroeder, 1956 and earlier refs.) in urine (Eades, Pollack and King, 1954; Schneider and Frahm, 1955) in blood (Hudson and Lombardo, 1955) and in liver (Butenandt, 1956) and chorion-gonadotrophic extracts (Schneider and Frahm, 1956; Schneider and Birtel, 1956). These discoveries prompted Schroeder and Voigt (1958) to investigate the efficacy of glycyltestosterone in the survival test on adrenalectomised golden hamsters, but the compound was inactive and also devoid of androgenic properties (Overbeck, 1957). Nevertheless certain steroids in the form of amino-acid esters do appear to retain their activity (Organon, 1960), and latentiation of hydrocortisone by conversion to the more soluble diethylaminoacetate hydrochloride has been used in dermatology (Welsh, 1956; Kuhn, 1959). Prednisolone and dexamethasone have also been administered as their diethylaminoacetates (Dorner and Hohlweg, 1961; Zicha and others, 1960). The β-diethylaminoethyl ester of dehydrodesoxycholic acid was found to have one-quarter of the potency of dehydrocholic acid as a choleretic agent on a molar basis (Gunter and others, 1950).

Recently interest has been aroused in the preparation of steroidal nucleotides like XVIII (Oertel and Agashe, 1960; Riess and Ourisson, 1961) and in the preparation of the nicotinic esters of male, female and adrenocorticoid hormones (Weichsel and Zirm, 1961), the synthesis of



XVIII

the latter being inspired by the fact that favourable analgesic activity was retained in the bisnicotinic ester of morphine (Skursky, 1957).

An important example of drug latentiation in the steroid field is provided by the general anaesthetic hydroxydione which is the sodium salt of the hemisuccinate of 21-hydroxypregnane-3,20-dione. In this compound the hemisuccinate moiety confers increased water solubility on a predominantly lipid soluble molecule and rapid hydrolysis by the non-specific esterase of the serum regenerates the parent compound which then crosses the blood-brain barrier and quickly builds up an anaesthetically active concentration in the brain (Figdor and others, 1957; Jakoby and Tomkins, 1956). There would seem to be no *a priori* reason why other readily hydrolysed water-solublising groups could not be employed in place of the sodium hemisuccinate moiety and indeed a series of substituted aminoacetates, tertiary amine hemisuccinate salts and a substituted ammonium phosphate salt of 21-hydroxy-pregnane-3,20-dione have been tested for anaesthetic potency (Fidgor and others, 1957). Also included in the study were the hemisuccinates of two 3-spirothiazolidine derivatives of 21-hydroxy-20-oxo-5α-pregnane. The results showed that the nature of the solubilizing group could markedly influence the activity displayed. The aminoacetates exhibited high potency and high toxicity. Within the series there was much variation in the time of onset of anaesthesia.

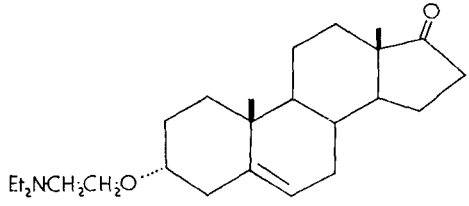
Although anaesthetic properties are associated with many steroids (Figdor and others, 1957; Selye, 1942) the phenomenon appears a structurally specific one as nuclear substitution profoundly alters activity (Figdor and others, 1957). Moreover if the action was simply an extension of the properties of the gaseous general anaesthetics whose activity can be related with their thermodynamic activities and hence with their concentration in the central nervous system (Ferguson, 1939), one would expect other predominantly lipid soluble molecules of intermediate molecular weight to form a connecting bridge between the simple anaesthetics on the one hand and the steroids on the other. The sodium hemisuccinates of representative mono- and di-terpenoids, however, proved inactive (Ahmad and others, 1961).

Little attention would appear to have been given to the possibility of improving the solubility, absorption and transport properties of nitrogenous drugs by forming inclusion compounds with desoxycholic acid, although it has long been recognised that various alkaloids do form choleic acids (Wieland and Sorge, 1916)—that is, inclusion compounds with desoxycholic acid. Inclusion compounds in which steroid hormone molecules are the entrapped species have, however, excited some interest and recently investigations of this kind of compound have been made with phenylurethane and hippuric acid as models for the study of the interaction of proteins with steroids (Dirscherl and Gerhards, 1961).

Supporting Moiety Theory

Nitrogenous steroids played an important part in the development of the supporting moiety theory which contends that the molecules of pharmacologically active substances consist of a radical moiety determining the

type of activity displayed and a supporting moiety conferring affinity for the site of action. Cavallini and his colleagues who were the first to formally state this theory (Cavallini, 1955; Cavallini and Massarani, 1959) employed such compounds in their early experiments. The actual compounds included the β-diethylaminoethyl ethers of oestrone, testosterone and 3α-hydroxy-17-oxoandrost-5-ene (XIX), and the bis β-diethyl-

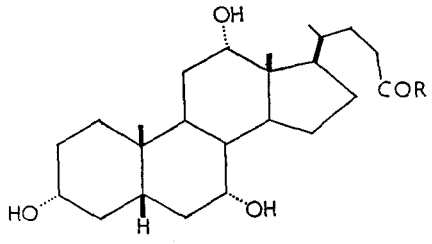


XIX

aminoethyl ethers of oestradiol and 3,17-dihydroxyandrost-5-ene (Cavallini and Massarani, 1951b). The combination of "stripped down" drug molecule (Gero and Withrow, 1957) or radical moiety (diethylaminoethanol) and the steroidal supporting moiety produced drugs with potent coronary vasodilatator properties (Cavallini and Massarani, 1951b, 1959) whilst the bisquaternary salts derived from the two di-ether compounds showed curare-like properties (Cavallini and Massarani, 1959; Cavallini and others, 1951). These quaternary salts also showed *in vitro* anticholinesterase activity (Cogni and Salvaneschi, 1951). Quaternary salts derived from the mono-diethylaminoethyl ethers exhibited ganglion-blocking activity (Cavallini and Massarani, 1959).

Coupling to the predominantly lipid soluble steroid nucleus would be expected to confer upon a radical moiety not only different solubility characteristics but perhaps also more favourable adsorption properties. Since the plasma proteins are of such a nature as to readily bind cholesterol, it is conceivable that such compounds could use an existing transport mechanism.

Application of these ideas was independently made by several groups of workers in the sulphonamide field, who attempted to overcome the unfavourable lipid solubility characteristics of this group of antibacterials by preparing sulphonamido derivatives of cholesterol (Lieb, 1947; Kwartler, 1948) or the bile acids (Redel and others, 1951; Haslewood, 1941). Sulphacholazine (XX,R = NH·NH·SO_2·C_6H_4-*p*-NH_2) was found



XX

to possess *in vitro* activity against streptococci and moreover on intravenous administration to rabbits it was demonstrated to gain access to the bile (Barber, Dible and Haslewood, 1943). It was unfortunate that the compound showed little activity against the coliform group of organisms. More recently steroidal 4-amino-2-methoxyphenyl ethers have been prepared with the object of using the bile acid transport system to bring schistosomicidal amines in contact with the adult schistosomes residing in the portal veins (Davis, 1962).

The N^1-hydroxycholanyl-*p*-aminophenylsulphonamides (e.g. XX,R = $NH{\cdot}SO_2{\cdot}C_6H_4$-*p*-$NH_2$) are claimed to exhibit antibacterial and antiviral properties (Berczeller, 1948) but where the sulphonamide moiety is attached directly to the steroid nucleus as, for example, at position 7, the compounds show little or no activity (Redel and others, 1951).

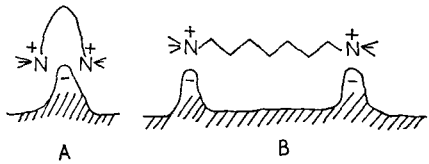
The rigid nucleus of steroids which possess a benzenoid ring A or a trans A/B ring junction is of interest as a supporting moiety from another point of view, since it can function as a skeletal framework upon which two or more radicals can be held in fixed spatial relationship to one another. Such a function for the steroid nucleus has been suggested to be involved in the activity displayed by the natural oestrogens where the oxygen functions at C(3) and C(17) are held at a rigid intergroup distance (see for example: Fisher, Keasling and Schueler, 1952; Macovski and Georescu, 1946; Oki, 1952). Other supporting moieties would be expected to be able to fulfil this role and in this may lie at least a part explanation for the potent oestrogenic properties displayed by such chemically diverse molecules as certain isoflavones, chlorotrianisene, the oestrogenolic acids and the oestrogenic stilbenes. Relatively little success has been achieved in securing alternative supporting moieties to replace the steroid nucleus in the androgenic and corticosteroid fields, although several attempts have been made (e.g. Clarke and Martini, 1959).

The hypothesis, first advanced by Paton and Zaimis (1949, 1951), that bisquaternary ammonium neuromuscular blocking agents interact by a two point attachment with anionic sites, normally involved in the physiological functioning of acetylcholine, has led to a number of attempts to define closely the actual interonium distance at the time of drug-receptor complex-formation. Unfortunately most compounds tested are conformationally non-rigid and so incapable of affording the desired information, as there is no reason to suppose that the thermodynamically most stable conformation of the isolated molecule is that actually adopted at the receptor. Whether various rigid bisquaternary ammonium salts in which the steroid nucleus functions as the supporting moiety will provide further information remains to be discovered. Although the receptor itself could conceivably be non-rigid, demonstration of activity in one rigid bisquaternary salt and absence of activity in another with a different interonium distance would represent a great advance.

Such steroidal bisquaternary ammonium salts will also be of interest in terms of Gill's hypothesis (1959) that completely rigid molecules should prove to be inactive due to variability in the receptors. This generalisation, which rests on the absence of ganglion-blocking activity in a limited

number of compounds, such as the completely rigid *NNN'N'*-tetramethyl-*p*-phenylenediamine dimethiodide (Wien and Mason, 1953) and certain virtually rigid furan derivatives still retaining a limited degree of rotational flexibility (Gill and Ing, 1958), certainly requires further substantiation. That there is a strong possibility that the molecules chosen do not possess a suitable interonium distance is indicated by the fact that other rigid molecules do indeed exhibit pronounced pharmacological activity. Examples are afforded by the natural oestrogens, testosterone, and the virtually rigid *cis* 1-hydroxy-2-trimethylammoniumcyclopentane, which shows marked depolarizing properties on the kitten phrenic nerve diaphragm (Standaert and Freiss, 1960).

Moreover, the two-point attachment theory is by no means universally accepted. Loewe and Harvey (1952) have postulated a one-point attachment theory in which the bulk of the molecule shields the receptor—the so-called "adumbration theory"—and their ideas have been extended by Fakstorp and others (1957). Again, conductimetric experiments have shown the extreme stability of the ion pair involving a single anion and a bisquaternary ammonium cation (Brody and Fuoss, 1956) which raises the possibility that the receptor-complex could be of type A, rather than type B (Cavallito and Gray, 1960).

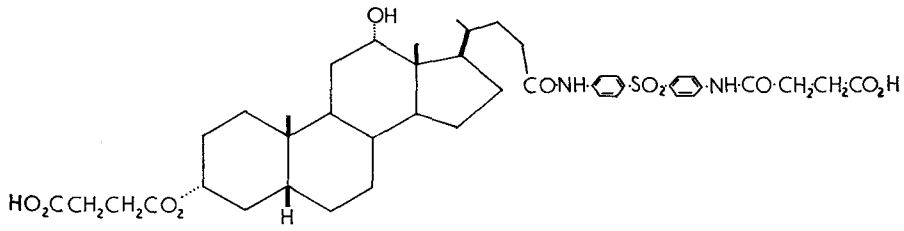


In view of all these facts, steroidal bisquaternary ammonium salts may well prove to be of great importance in distinguishing between the various possibilities.

It is to be noted that malouetine (II) is not fully rigid, due to rotation about the C(17)—C(20) bond and to the possibility of chair to boat conformational isomerism in ring A, and these effects permit some variation in the interonium distance.

The discovery that marked antituberculous properties were present in various 4,4'-diaminodiphenylsulphone derivatives, thiosemicarbazones and hydrazones, resulted in the synthesis of many related compounds, including several steroidal derivatives in which the steroidal portion can be regarded as a supporting moiety. Some of these compounds proved to have a high activity, especially the bile acid amide derivatives of diaminodiphenylsulphone (Berczeller, 1949; 1958). One compound in this series, 4-(3-hemisuccinyldesoxycholylamino)-4'-hemisuccinylamino-diphenyl-sulphone (XXI) also proved to be an active inhibitor of the multiplication of the PR8 strain of influenza virus A in the chick embryo (Berczeller, 1958–59).

The hydrazones and isonicotinylhydrazones of testosterone, oestrone and dehydroandrosterone all proved active against the tubercle bacillus *in vitro* (Cavallini and others, 1952; Mantegazza and Tommasini, 1952)



XXI

as did the thiosemicarbazones of testosterone, progesterone and dehydroandrosterone (Mantegazza and Tommasini, 1951). These derivatives are virtually devoid of the physiological properties of the parent steroids (Cavallini and Massarani, 1951a; Ercoli, Koller and de Ruggièri, 1951). Dehydroisoandrosteryl-thiolpyrazinoate which was prepared as an analogue of the antituberculous ethylthiolpyrazinoate proved inactive (Kushner and others, 1955) as did the benzoylhydrazone of cholestenone (Offe, Siefken and Domagk, 1952). Cholesteryl *p*-nitrobenzoate, unlike certain other esters of *p*-nitrobenzoic acid was inactive against pneumococci (Mayer and Oechslin, 1939).

The promise shown by the nitrogen mustards as potential anti-cancer agents inspired the utilisation of the steroid nucleus as a supporting moiety in this field as well, and several mono- and bis-(2-chloroethyl)aminosteroids have been synthesised (Burstein and Ringold, 1961; Gensler and Sherman, 1958; Havranek and Doorenbos, 1960). Only three of these compounds appear to have been tested for anti-tumour activity, however, and these proved inactive (Havranek and Doorenbos, 1960). *Added in proof:* A more recent investigation has shown that antitumour activity is present in certain steroidal nitrogen mustards (Rao and Price, 1962).

The potent positive inotropic cardiac activity present in both the steroidal cardiac glycosides and the erythrophleum alkaloids led two groups of workers to prepare steroidal analogues of the latter, in which bile acids were used to replace the diterpenoid acids as supporting moieties (Ruzicka, Plattner and Engel, 1944; Uhle, Mitman and Krayer, 1956), but the new compounds were virtually inactive. It will be interesting to see whether steroidal esters of pyrrole -α-carboxylic acid will be prepared as analogues of the diterpenoid alkaloid ryanodine (Valenta and others, 1962) which exhibits such a remarkable pharmacological action on muscle (Hillyard and Procita, 1959 and refs. cited).

Several groups of naturally-occurring nitrogenous steroids can probably be quoted as exemplifying the supporting moiety theory, although in some cases it is difficult to distinguish a supporting moiety function from a latentiation function. A good illustration of this situation is afforded by the taurine and glycine conjugates of the bile acids whose anions are the true bile salt anions. Another example is afforded by the readily hydrolysed veratrum ester alkaloids, such as protoveratrine, which is some 6,000 times as toxic on a molar basis as is its alkamine, protoverine (Krayer, Moe and Mendez, 1944).

It is also possible to consider the suberylarginine radical of the toad poisons as a supporting moiety, but the position with the cardiotonic steroids is particularly complex. It is generally held that the strong positive inotropic action is intimately linked with the unsaturated lactone function (Chen and Elderfield, 1940; Goodman and Gillman, 1955) but to regard this group as the active moiety is a gross oversimplification, as varying degrees of positive inotropic action are shown by the dihydrocardenolides (e.g. Jacobs and Hoffmann, 1927; Vick, Kahn and Acheson, 1957), the bile acids (e.g. Wakim, Essex and Mann, 1939), certain steroidal alkaloids (Benforado, 1957; Krayer, Moe and Mendez, 1944; Quévauviller and Blanpin, 1958), cortisone (Fleischhacker, 1956) and various other steroids (e.g. Abrams and Harris, 1951; Hajdu and Szent-Gyorgyi, 1952; Tanz and Kerby, 1961) none of which possess an unsaturated lactone. Indeed it is tempting to regard the lactone as a supporting moiety intensifying an activity associated with a hydroxylated steroid nucleus (Craig and Jacobs, 1943). A supporting moiety role can probably be assigned to the sugar residues of the cardiac glycosides since they have a marked influence on distribution and solubility properties and so affect the time of onset and duration of action (Chen, Henderson and Anderson, 1951; Keyl and Dragstedt, 1954). Although some scilladienolide genins show some potency in bioassays, the duration of action is transitory and so it is concluded that the sugar moieties play an indispensable role in determining the activity of the glycosides (Stoll, 1956). The effect of varying the number and nature of the sugar residues on the activity displayed has been summarised in several places (Chen, 1945; Fieser and Fieser, 1959; Oettel, 1947; Tamm, 1957).

The pharmacological actions of the suberylarginine conjugates of the bufadienolides were studied by Gessner (1926) and his work was followed by an elegant series of papers by K. K. Chen and his colleagues (e.g. Chen and Chen, 1934). The results of these and other studies (Arora, 1953; Chen, Anderson and Rose, 1952) would indicate that the compounds possess broadly similar pharmacological properties to the cardiac glycosides, but that the suberylarginine moiety does affect the rate of penetration into and removal from the myocardial tissue, thus producing differences of a quantitative nature.

Other Theoretical Interests of Nitrogenous Steroids

Several isolated examples are known in which nitrogenous steroids have played minor roles in studies designed to throw more light on the intimate nature of biological processes. One such instance involves the application of complex steroid derivatives in the study of artificial antigens (Grob and Goldberg, 1949).

The interesting hypothesis has put forward that certain steroids may interact with enzymes and other proteins by formation of spirothiazolidines since 3-oxo-steroids lacking a 4,5-double bond were shown to form such compounds under a variety of conditions with cysteine (Lieberman, 1946), but this suggestion is in need of further substantiation.

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