REVIEW ARTICLE

STEREOISOMERISM AND BIOLOGICAL ACTION

By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C. and
A. F. Casy, B.Sc., F.P.S., A.R.I.C.

*Chelsea School of Pharmacy, Chelsea Polytechnic, London, S.W.*3

The relation of chemical structure to biological activity has long been the subject of extensive chemical and pharmacological investigations. That stereochemical factors also play an important role in the determination of biological action is evident from the existence of so many stereoisomeric pairs of compounds in which the members of each pair differ considerably in the magnitude of their biological effects. The purpose of this review is to discuss the features which can result in biological discrimination between isomers, and to present information concerning classes of compounds in which activity is greatly dependant upon stereochemical factors.

Stereoisomers are defined as isomeric substances which differ only in the geometrical arrangement (configuration) of their atoms or groups. Stereoisomerism may be subdivided into optical and geometrical isomerism.

Optical isomerism

Optical isomers (enantiomorphs) may be defined as stereoisomers in which the atoms or groups comprising the compound are arranged in two different ways to form two molecular species which differ from one another only as an object differs from its mirror image (see Fig. 1 in which A, B, C and D represent groups or atoms attached to a carbon atom (asymmetric carbon atom); (a) and (b) are thus enantiomorphs. Such isomers commonly arise from the presence of an asymmetric centre within the molecule, although this feature is not essential for molecular dissymmetry. Optical isomers differ in their action on plane-polarised light. They have identical chemical properties except in their reactions with other optically active molecules. Furthermore, the combination of enantiomorphs with another optically active substance gives two products (diastereoisomers) which are not related as object to mirror image, and may exhibit large differences in physical properties such as solubility, partition coefficients and reactivity. One other important feature arises from the difference in the arrangement of groups in enantiomorphs as follows. If three of the groups attached to an asymmetric carbon atom are aligned towards a particular surface, then

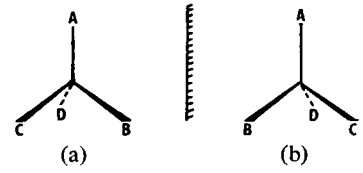
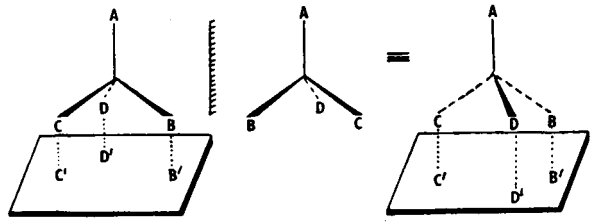


FIG. 1. —— represents in the plane of the paper, ▬▬ represents pointing towards and – – – represents pointing away from the observer.

a similar alignment of the same three groups is not possible in the enantiomorph (see Fig. 2). The value or direction of the optical rotation of enantiomorphs has almost no significance from a configurational and thus an "alignment to a receptor surface" point of view.

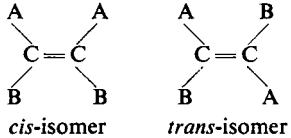


(a) (b) (b)

FIG. 2. Alignment of enantiomorphs a and b to a receptor surface. C, D and B represent groups in the enantiomorphs and C′ D′ and B′ represent their points of alignment at the surface.

Geometrical isomerism

Geometrical isomers may be defined as stereoisomers in which the molecular species are not related as object to mirror image. This type of isomerism may result when rotation within the molecule is restricted, for example by double bonds (Fig. 3) or by rigid (or semi-rigid) ring systems (Fig. 4). Usually, certain groups in a geometrical isomeric pair of compounds are separated by different distances.



cis-isomer *trans*-isomer

FIG. 3

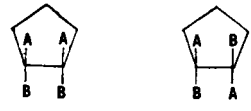
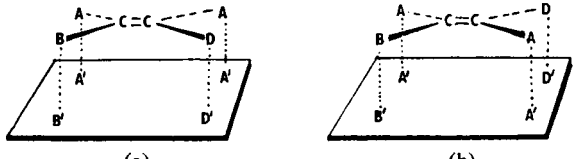


FIG. 4

Geometrical isomers may differ greatly in physical properties such as solubilities, partition coefficients, dissociation constants, etc. Frequently, differences in chemical properties are also very marked, especially those involving the participation of the groups which are separated by different



(a) (b)

FIG. 5. Alignment of geometrical isomers a and b to a receptor surface. A, B and D represent groups in the isomers and A′, B′ and D′ represent their points of alignment to the surface.

distances in the isomers., e.g., maleic acid with two carboxyl groups in proximity to each other readily forms an anhydride whereas its geometrical isomer, fumaric acid, does not. The difference in the relative positions of certain groups results in the failure of geometrical isomeric

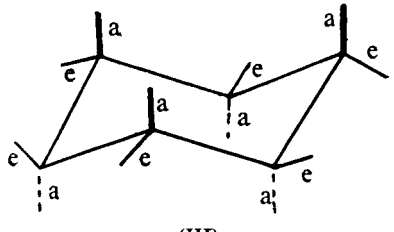
pairs to orient such groups similarly to a receptor surface. For example, if the *cis*-isomer (A groups *cis*) (Fig. 5a) is held at a particular surface by forces involving both A groups, then the *trans*-isomer (*b*) cannot present these groups in a similar manner, and differences in the adsorption of the isomers will be apparent. However, both the isomers shown can present groups A and B in similar ways. It follows that a "three-point" reception of three of the four groups will involve discrimination in the adsorption of the isomers.

Conformational considerations

In recent years, a further aspect of stereoisomerism which has important implications in the observed differences of the biological activities of geometrical isomers has received considerable attention, namely the conformational aspect (see Barton[1], Klyne[2] and Orloff[3] for detailed accounts). Conformational analysis is the study of the different arrangements in space of atoms or groups in a single classical organic structure (configuration), e.g., the chair (I) and boat (II) conformations of *cyclo*hexane. The energy barriers between the various conformations are not sufficiently high, in the examples so far examined, to allow of the isolation of two separate conformations of the same classical configuration.

In general, for the 6-membered carbocylic or heterocyclic ring structures, the chair conformation is the more stable one. This obtains because, although the boat and chair forms are equally strain free in the classical sense, the mutual repulsions of neutral non-bonded atoms results in the chair form being the more stable since the distance between the non-bonded atoms is at a maximum. The C—H bonds of the chair conformation of *cyclo*hexane are of two different types, 6 bonds which lie approximately in the general plane of the ring and are designated equatorial (e) bonds, and 6 bonds which are perpendicular to the general plane of the ring, 3 pointing in one direction and 3 in the other; these are designated axial (a) bonds (see III).

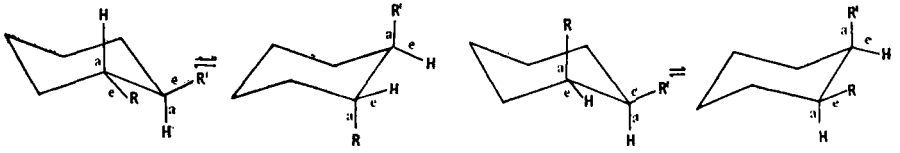
(I) (II)



(III)

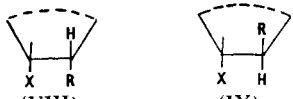
In the absence of strong electrostatic effects, the most stable conformation of a molecule composed of six-membered alicyclic rings will be built up of chair forms with the larger groups in equatorial positions, e.g., a *trans*-1:2-disubstituted *cyclo*hexane isomer will have the chair conformation (IV) rather than the alternative chair form (V) in which the non-bonded interactions are greater. [Conformations (IV) and (V) can be interconverted by the rotation and twisting (not the breaking of bonds).]

A *cis*-1:2-isomer, in which R′ is a larger group than R, will have the conformation (VI) rather than (VII).



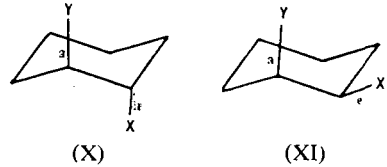
(IV) (V) (VI) (VII)

Many facts concerning the thermodynamic stability and the reactivity of epimeric alicyclic compounds (i.e., geometric isomers in which inversion occurs by the interchange of positions of an H atom and another group —see (VIII) and (IX)—can be explained in terms of the equatorial and axial conformations of the groups concerned. For example, equatorial hydroxyl groups are thermodynamically more stable than axial ones, and when a polycyclic secondary alcohol is heated with alkali ("equilibrated"), the isomer with an equatorial hydroxyl group is present in larger amount in the reaction mixture; ecgonine (axial carboxyl group) is isomerised by alkali to ψ-ecgonine (equatorial carboxyl group). However, in a consideration of the possible explanation of the differences in biological actions of isomers, the effects of conformations upon the course and rate of reactions are of greater interest. The differences in the reactions of various conformations are due to differences in the accessibility of the groups concerned, or differences in the steric requirements of the reactions which may be satisfied to a greater or less extent by the particular conformation.



(VIII) (IX)

A few of the observed differences in reactions of axial and equatorial groups which may explain differences in biological action of certain isomers are here enumerated. Axial groups are subjected to greater steric hindrance (from the axial hydrogen atoms or groups on the β-carbon atom) than the corresponding equatorial ones. This is reflected in the following differences in reactions: equatorial hydroxyl groups are more readily esterified than the corresponding axial ones; equatorial acyloxy groups are more readily hydrolysed than axial groups; equatorial carboxyl groups are more readily esterified and the product more readily hydrolysed than the corresponding axial groups; axial secondary alcoholic groups are more readily oxidised (attack upon the C—H bond is the rate determining step) than corresponding equatorial groups.



(X) (XI)

The observed differences between the reactions of various conformations upon the demand of a reaction with steric requirements is illustrated in the following example. Bimolecular elimination reactions will only proceed readily if the four centres of importance in the reaction lie in one plane. This condition is satisfied by a 1:2-*trans*-disubstituted *cyclo*hexane in which both groups are axial or are able to adopt the

axial conformation (X), but not by the corresponding *cis*-compound (XI), in which one group must necessarily be axial and the other equatorial.

Before considering the implications of the above differences between enantiomorphs and between geometrical isomers and their various conformations upon biological activities, a brief consideration of the various factors which may influence the latter activities is included (see Albert[4], Sexton[5] and Danielli[6] for a detailed treatment).

Biological Action of Molecules

Biologically active compounds may be broadly classified into those which are *structurally non-specific* and those which are *structurally specific*. The first type have a general non-selective action upon tissues or enzyme systems, e.g., the depressant action of chloroform, bromoform and trichloroethylene; compounds of this type usually can give the same biological response although differing greatly in chemical constitution—their mechanism of action is probably a physico-chemical one. The second type probably act at specific receptor sites in tissue or enzyme systems to form reversible complexes, the dissociation constants of which are affected by the closeness of fit of the drug to the receptor.

The biological response upon the presentation of a reagent to a living organism is dependent upon (*a*) the access of the molecule to the site of action, and (*b*) the reaction of the molecule at the site, and many factors may influence the result. Some of the more important of these factors which may be affected by stereochemical features of molecules are given below.

Penetration of membranes. The membranes surrounding all cells are composed of layers of lipoids and proteins and are strongly charged. Ions, because of their charge and their relatively greater size due to hydration, penetrate these membranes less readily than the corresponding neutral molecules.

Adsorption at surfaces. Adsorption may be indiscriminate or specific. Adsorption of soaps which are adsorbed on any surface irrespective of its chemical nature is an example of indiscriminate adsorption; in this type, neutral molecules are usually held more firmly than ions. The specific type of adsorption usually involves the mutual attraction of unlike charges (cation for anion and *vice versa*) reinforced by the short range van der Waals forces which require close fitting complementary surfaces to be effective.

Chemical reactivity. Not only will this factor influence the attack upon tissue or enzyme system by which a biological response may be mediated, but it will affect the metabolism and the distribution of a molecule within an organism.

Degree of ionisation. At a given *p*H value, the proportion of ions to neutral molecules is dependent only upon the pKa of the ionisable groups. Because ions and neutral molecules usually behave differently in their penetration of membranes, their adsorption at surfaces, their lipoid solubility and sometimes their chemical reactivity, any stereo-chemical factor which can influence the pKa of a group of a molecule which is partially

ionised at physiological *p*H may have a profound effect upon the biological action.

Lipoid solubility. This factor will not only influence the penetration of membranes by a reagent, but will influence the degree of localisation of a substance. It has a great effect especially upon structurally non-specific agents.

Steric factors. Such factors can influence the chemical reactivity of a group within the molecule and affect especially the specific adsorption of molecules at surfaces.

Differences in Behaviour of Stereoisomeric Pairs of Compounds

The individuals of stereoisomeric pairs of compounds can exhibit features which may influence the above factors differently and so lead to a discrimination in the biological responses. The differences in the summation of the above factors for the isomers may be considered as leading to (1) differences in the distribution of the isomers, (2) differences in the properties of the isomer-drug receptor combination and (3) differences in the strength of attachment or "fit" of the isomers to a complementary drug receptor surface.

1. *Differences in the Distribution of the Isomers*

Two isomers which inherently possess identical biological effects if presented in equal concentrations to the site of action, will give an overall difference in biological response in the whole organism if concentration differences are produced before the molecule arrives at the site, whether the compound be structurally specific or structurally non-specific.

Optical isomers. A difference in the distribution of enantiomorphs may result from their combination with another optically active substance to give diastereoisomers of differing solubilities which will affect the penetration of membranes or the solubilities in various tissues.

Distribution will also be affected by preferential destruction or metabolism of one of the enantiomorphs by a dissymmetric enzyme system, e.g., after the administration of racemic mepacrine, optically active mepacrine can be detected in the urine[7]; (−)-5-ethyl-5-phenylhydantoin is stated to have a more powerful "anæsthetic" action than the (+)-isomer[8] and this may result from the greater rate of metabolism of the latter since it has been shown that it disappears more rapidly from rat plasma than its enantiomorph[8]; enzymes which are present in pig and rat kidney hydrolyse the acyl-L-amino-acids much more readily than their enantiomorphs[9].

It is also possible to explain the above observations in terms of preferential adsorption (or absorption) of one isomer at a surface in the organism (see later) leading to concentration differences. This phenomenon is well known *in vitro*. Porter and others[10] showed (+)-isomers of certain dyes were held more strongly by wool than their enantiomorphs. More recently, Bradley and Easty[11] have shown that wool and casein selectively absorb (+)-mandelic acid and (+)-α-naphthylglycollic acid from aqueous solutions of their respective racemic mixtures. Resolution of (±)-*p*-phenylenebisiminocamphor and "Tröger's base" by the selective adsorptive action of lactose has been reported[12,13].

Geometrical isomers. Large differences in physical and chemical properties exhibited by geometrical isomers can readily affect their distributions. Explanations of many of the former differences can be given in terms of spatial separation of groups or accessibility of groups and it may well be that these effects can be correlated with discriminations in biological responses. For example, the fact that the pKa_1, of ψ-ecgonine is greater than that of ecgonine, but the pKa_2 of ψ-ecgonine is lower than that of ecgonine can be explained in terms of the closer proximity of the amino and carboxyl groups in ψ-ecgonine[14]. Such differences could have great effects upon the distribution of geometrical isomeric compounds if the isomers were partially ionised at physiological *p*H.

The difference in the conformation of epimers with its consequent effect upon the rate of chemical reactivities could also result in concentration differences, e.g., *epi*cholesterol (axial-OH) is more readily oxidised than cholesterol (equatorial—OH)[15]; only those cyclitols with axial hydroxyl groups are oxidised by *Acetobacter suboxidans*[16].

Geometrical isomers, unlike optical ones, can readily be separated by adsorption on optically inactive materials, e.g., chromatography upon alumina. Consequently, differences in the adsorption of the isomers could lead to concentration differences at sites of action. The differences in the adsorptive forces upon the isomers is sometimes explicable in terms of conformations of groups, e.g., in the chromatography of steroids it has been shown that the epimers with an equatorial hydroxyl group are adsorbed more strongly than the corresponding axial epimers[17].

2. *Differences in the Properties of the Drug-receptor Combination*

Many biological responses are now attributed to the reversible "combination " of the molecule in question with one or more "receptors" in tissue or enzyme system. It is not implied that combination alone produces the response, but that a suitable combination may initiate, modify or block a series of interdependent chemical processes. Ionic forces, hydrogen bonding and van der Waals forces are probably involved in the "combination" of drug and receptor.

Optical isomers. It is possible for enantiomorphs to be held equally strongly to a receptor site (e.g., Fig. 1—assume that the groups C and B only involved in the "combination," or alternatively C, B and D but that the receptor "combines" with D equally strongly although differently orientated to the receptor surface in the two isomers) and yet nevertheless to exhibit differences in biological action due to the differences in properties of the two drug-receptor combinations. This explanation of the difference in effects of enantiomorphs was developed by Cushny[18]. He suggested that the two combinations were analogous to diastereoisomeric forms and thus would be expected to exhibit different properties of solubility, etc., as for instance the combinations of (+)- and (−)-tartaric acid with (+)-cinchonidine. In the case of the constrictor action of (−)- and (+)-adrenaline on the vessels of the conjunctiva, it may be considered that the more active (−)-isomer readily produces a

reaction at the neuro-effector cell junction, whereas the less active (+)-isomer only does so when present in a higher concentration than the former[18].

The wide differences in properties exhibited by geometrical isomers renders unlikely the possibility that they could be held equally strongly at a receptor site, and consequently the difference in properties of the drug-receptor combination is unlikely to be a factor worthy of consideration in an explanation of any observed discrimination in biological response.

3. *Differences in the "Fit" to a Complementary Receptor Surface*

It is possible to account for the difference in biological activities of certain enantiomorphs in terms of a "three-point" combination of groups attached to an asymmetric carbon atom with three areas of the receptor surface. Figure 2 illustrates the fact that only one enantiomorph could present the three groups in the correct relative positions; the other isomer could only have two groups correctly aligned. All the groups do not need to be ionic or reactive, e.g., the "three-point" attachment groups for analgesic drugs have been postulated[19] as a flat aromatic ring, a basic group (ionised) and a projecting hydrocarbon moiety.

The differences in the biological effect of enantiomorphs in a series of compounds only becomes of established significance in terms of alignment to areas in a specific receptor surface, if the configurations of the biologically more active enantiomorphs of the series are shown to be identical.

It is possible that the biological discrimination between enantiomorphs can be accomplished in a number of ways involving the presentation of three "groups" to the surface, and these are outlined below.

1. All three groups are essential for the binding of the molecule to the receptor site, the areas of the latter being so orientated that only one enantiomorph fits correctly. It follows that the other enantiomorph will be inactive, and since it does not "fit" the surface, will not antagonise the action of the active one even if presented in higher concentrations than the latter.

2. Three groups are involved, but the intensity of the biological action is dependent solely upon the ease of combination of the enantiomorphs with the receptor; one enantiomorph "fits" better than the other. This hypothesis was emphasised by Easson and Stedman[20]. The less active one will not antagonise the biological effect of the more active one. (However, if the nature of the drug-receptor complex is also involved in the biological action, it would be possible for the lesser active enantiomorph, in high concentrations, to antagonise the more active one in lower concentrations). It is possible that slight modifications of the highly active isomer might yield compounds of antagonistic action because they could fit the surface correctly, but fail to evoke the reaction sequence which gives the particular biological response. Assume, for

example, that an N—Me group was one of the groups involved and demethylation occurred in the reaction. An N—Allyl derivative of the active enantiomorph could "fit" the receptor correctly but would fail to evoke the reaction sequence and, in suitable concentrations, could block the site and thus act as an antagonist. A similar derivative of the less active enantiomorph would act only as a weak antagonist. (See Fig. 6).

3. Two groups are directly involved in the "combination" of the molecule with the receptor (Fig. 7a and b; C and B), and the "third group" (Fig. 7a and b; Me), improves the combination when correctly,

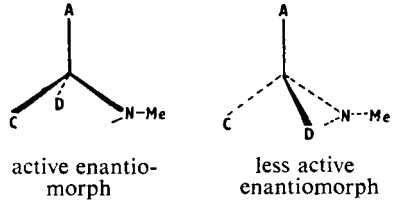
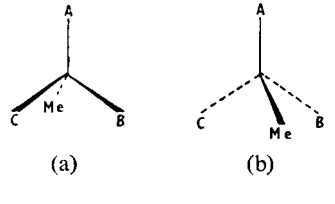
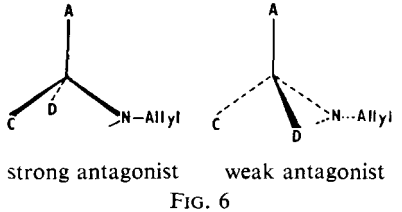


FIG. 6





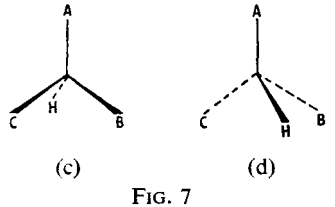


FIG. 7

but hinders when incorrectly, orientated; consequently the isomers have different activities. It is unlikely that the less active isomer would antagonise the activity of the more active one. The replacement of the "third group" by one of the same type which did not alter the physical properties such as lipoid solubility or dissociation constant of the molecule, e.g., replacement of $-CH_3$ by $-H$ or $-C_2H_5$ in certain circumstances, would be expected to yield compounds (Fig. 7c and d) with activities less than the more active enantiomorph, but greater than the less active one.

It is recognised that the above subdivisions of the type of "three-point" reception are somewhat arbitrary. In practice, the actual mechanism involved in the mediation of the biological effect may result from a combination of the above types to a greater or lesser extent, complicated by the effect of the nature of the drug-receptor combination.

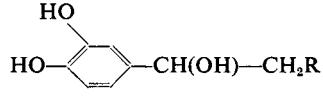
Geometrical Isomers. In Figure 5 it was shown that there were differences between the isomers in the orientation of groups to a receptor surface if three groups were involved. Consequently, arguments apply similar to those used above. The differences in the distance between two groups in the isomer can also lead to discrimination if these have to "fit" two centres in the receptor, e.g., Baldridge *et al.*[21] have used the

enzymatic responses to *cis*- and *trans*-isomers in the *cyclo*hexane series to provide information concerning the distance between the esteratic and anionic sites of the acetylcholinesterase surface.

In the remainder of this review, attention will be given to series of compounds, the members of which exhibit a common biological action which has been shown to be greatly dependent upon the stereochemical features of the molecules concerned.

Sympathomimetic Agents

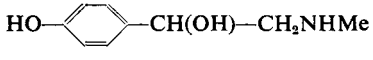
Sympathomimetic substances mimic the effects of stimulation of the sympathetic nerve fibres. An important member of the group is adrenaline (XIIa) which possesses one asymmetric carbon atom and occurs naturally as the (−)-isomer. The (+)-form, obtained by synthetic methods, has been shown to be from 12 to 20 times less active than the (−)-isomer[22]. The relative effects upon systolic blood pressure of the enantiomorphs of both noradrenaline (XIIb) and isoprenaline (XIIc) have been determined and, in each case, the (−)-isomer is found to



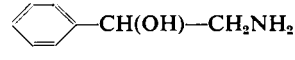
(XII)

R = —NHMe (a), —NH_2 (b), —NH*iso*C_3H_7 (c)

possess the greater activity[23,24]. Replacement of the 3:4-dihydroxyphenyl moiety of adrenaline by the 4-hydroxyphenyl or the phenyl group leads to a reduction in pressor activity. In two compounds of this type (XIII, synephrine, and XIV), the (−)-isomers are again found to be more active than their antimers[25,26].



(XIII)



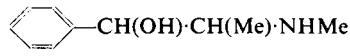
(XIV)

Easson and Stedman[20] accounted for the different activities of the adrenaline antimers in terms of a difference in their ease of attachment to a receptor surface. They suggested that only the (−)-isomer can come into complete contact with the surface, and that the (+)-isomer behaves as if one of the groups necessary for maximum activity is missing. In adrenaline and related compounds, three structural features appear to be essential for maximum pressor activity, namely, a basic centre, a phenyl group and an alcoholic hydroxyl group. The above hypothesis is supported by the fact that (+)-adrenaline has an activity approximating to that of desoxyadrenaline, in which one essential group, the alcoholic hydroxyl group, is missing. Further support has been provided by Schaumann[27] who found that the (+)-isomer of corbasil [1-(3:4-dihydroxyphenyl)-2-aminopropan-1-ol] has approximately the same activity as desoxycorbasil [1-(3:4-dihydroxyphenyl)-2-aminopropane], both compounds being about 160 times less active than (−)-corbasil.

Furthermore, both the (+)-isomer and the desoxy-compound exhibit the same qualitative differences from (−)-corbasil.

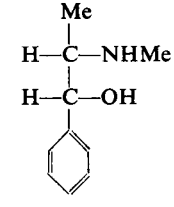
Although configurational studies have not been carried out in this series, Dalgliesh[28] has presented much indirect evidence indicating that (−)-adrenaline and (−)-noradrenaline possess the same configuration.

Ephedrine (XV) and related compounds possess pharmacological actions that are similar to those of adrenaline but which differ in duration and mechanisms of action. The stereochemistry of ephedrine is more complex than that of adrenaline in that the former molecule possesses one additional asymmetric carbon atom and exists in two diastereoisomeric forms (ephedrine and ψ-ephedrine). As with adrenaline, there are marked differences in the biological activities of the various enantiomorphs[29]. Natural (−)-ephedrine is approximately 3 times as active as the (+)-form, whereas the (+)-form of ψ-ephedrine possesses 7 times the activity of its antimer. The (−)-form of norephedrine has 1·5 times the pressor activity of the (+)-isomer, the enantiomorphic ψ-norephedrines being equal in activity[30,31].

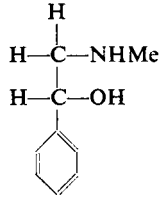


(XV)

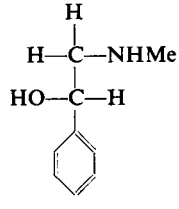
Considerable investigations have been carried out to establish the configurations of the two asymmetric centres of the enantiomorphs of this series[32,33]. It is significant that those enantiomorphs which exhibit the greater pressor activity possess identical configurations with respect to the carbon atom bearing the basic group. On the basis of the *lævo*-rotation of both natural adrenaline and (−)-ephedrine, Freudenberg[34] assigned the configuration (XVI) to (−)-adrenaline. Dalgliesh[28], however, from indirect chemical evidence, considers (−)-adrenaline to be related in configuration to L-(+)-mandelic acid (XVIII) and thus possesses the opposite configuration to that proposed by Freudenberg.



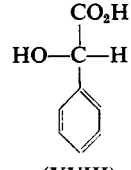
(−)-ephedrine



(XVI)



(XVII)

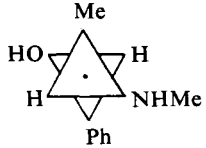


(XVIII)

If configuration (XVII) be subsequently confirmed, the difference between the configurations at the hydroxyl bearing carbon atom of (−)-adrenaline and (−)-ephedrine may be of significance in the interpretation of the pharmacological results.

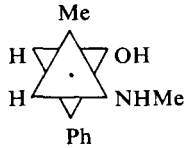
Fodor *et al.*[35] have shown the rate of acyl-migration in *N*-benzoyl-(±)-ψ-ephedrine to be far greater than the rate in the corresponding ephedrine compound. They conclude that, in the latter compound, the —OH and —NHMe groups are relatively distant from one another, whereas in ψ-ephedrine they are relatively close. Similar results were obtained, and like conclusions drawn, for norephedrine and ψ-norephedrine.

Close[36] has presented further chemical evidence to support these contentions and has proposed (XIX) and (XX) as the most probable conformations of (−)-ephedrine and (+)-ψ-ephedrine that represent the resting states of the molecules.



(−)-ephedrine

(XIX)

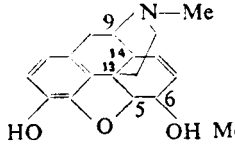


(+)-ψ-ephedrine

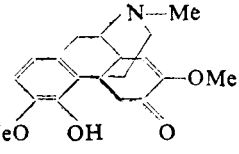
(XX)

Analgesics

(*a*) *Morphine and related compounds.* The structure of morphine (XXI) proposed by Gulland and Robinson[37] and recently conclusively confirmed by synthesis[38], possesses five asymmetric centres ($C_{(5)}$, $C_{(6)}$, $C_{(9)}$, $C_{(13)}$ and $C_{(14)}$). The naturally occurring (−)-isomer has not been compared in activity with its (+)-enantiomorph, now potentially available by the synthesis of Gates and Tschudi[38]. Certain morphine derivatives have, however, been obtained in their enantiomorphic forms by synthesis



(XXI)

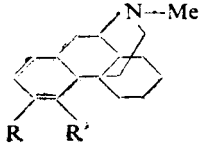


(XXII)

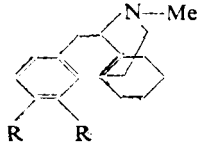
from the alkaloid sinomenine (XXII), a substance which, apart from the absence of the oxygen bridge, possesses a ring system identical to that of morphine. Moreover, the configurations of the asymmetric centres $C_{(9)}$, $C_{(13)}$, $C_{(14)}$ and $C_{(5)}$ (when generated), are the reverse to those of morphine, and on this account, sinomenine affords a route to substances that bear a mirror image relationship to the corresponding morphine derived compounds. Thus Takebe and Kitasato[39] have prepared (+)-dihydrocodeinone, (+)-dihydrothebainone, (+)-tetrahydrodesoxycodeine, (+)-dihydrothebainol, (+)-1-bromo-sinomeninone and (+)-dihydrosinomeninone, all of which are found to be active convulsants that do not show the analgesic action of the corresponding morphine derivatives. Goto and Arai[40] report (+)-dihydromorphine, derived from sinomenine, to be equally powerful a narcotic as morphine, but do not specify its analgesic properties.

(*b*) *Morphinan and related compounds.* Racemic *N*-methyl-morphinan (XXIII; R = R′ = H), prepared by Grewe and Mondon[41] by cyclisation of compound (XXIV; R = H), represents a synthetic analgesic that possesses the same molecular skeleton as morphine. Furthermore, its steric identity with morphine has been shown by Grewe *et al.*[42] in the synthesis of tetrahydrodesoxycodeine (XXIII; R = OMe, R′ = OH) by cyclisation of compound (XXIV, R = OMe), the (−)-isomer being identical with (−)-tetrahydrodesoxycodeine, prepared from codeine. The relative activities of the enantiomorphic forms of the potent analgesic

3-hydroxy-*N*-methylmorphinan (Dromoran, racemorphan XXIII, R = OH, R′ = H), prepared by a similar cyclisation process, have been studied. The (−)-isomer (levorphan) has approximately the same toxicity but a higher analgesic action than the racemic compound, while



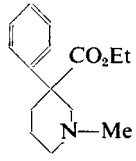
(XXIII)



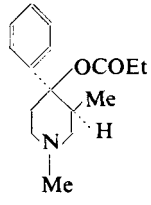
(XXIV)

the (+)-isomer (dextrorphan) is less toxic and inactive[43]. Levorphan is also a greater respiratory depressant than dextrorphan. The (+)-, (−)- and (±)-methyl ethers of Dromoran (XXIII, R = OMe, R′ = H) exhibit parallel analgesic characteristics, although they are less potent and more toxic than the parent compounds[44].

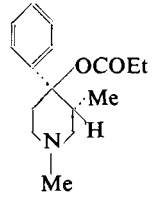
(*c*) *Pethidine and related compounds.* Pethidine itself is a symmetrical molecule but several asymmetric modifications have been obtained during



(XXV)



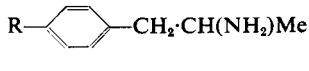
(XXVI)



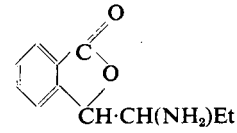
(XXVII)

the synthesis of pethidine type compounds. Macdonald *et al.*[45] reported that racemic nor-*iso*pethidine (XXV) is one quarter, and the (−)-isomer one half as active as pethidine, while the (+)-isomer is inactive. The highly active analgesic 1:3-dimethyl-4-phenyl-4-propionyloxypiperidine has been obtained in two geometrically isomeric forms, in which the propionyloxy and methyl groups are respectively *cis* and *trans* (configurations assigned only provisionally[46]). Pharmacological results on rats show the *cis*- form (XXVI) to be from 5 to 6 times more potent than the *trans*-modification (XXVII). Furthermore, the *cis*-form has been resolved and the (−)-isomer found to be more than twice as active as its antimer[47].

(*d*) *Aralkylamines.* A systematic investigation of aralkylamines by Fellows and Ullyot[48] resulted in several examples of asymmetric compounds in which the various forms showed differences in analgesic activity. (±)-Amphetamine (XXVIII, R = H) is found to possess weak analgesic properties while the (−)-isomer is inactive. The (±)- and (+)-forms of the corresponding *p*-hydroxy derivative (XXVIII, R = OH) possess marked analgesic properties, the (−)-isomer showing only slight activity. Ullyot and his colleagues[49] showed that 1-amino-1-phthalidylpropane (XXIX) possesses considerable activity. This compound,



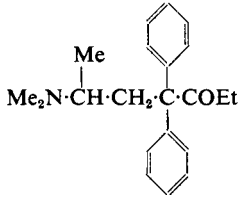
(XXVIII)



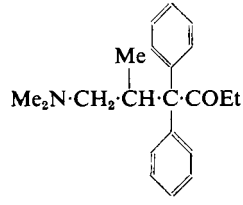
(XXIX)

which contains two asymmetric centres, was separated into two racemic mixtures, one of which was found to be more active than the other.

(*e*) *Methadone and related compounds.* Methadone (XXX) itself possesses one asymmetric centre and its resolution was first reported by Thorp, Walton and Ofner[50] who found the (−)-isomer to be the more active form. This fact was soon confirmed by other workers and the (−)-isomer shown to be approximately 20 times as active as the (+)-form[51,52]. The optical isomers of *iso*methadone (XXXI), obtained by Larsen *et al.*[53], show parallel differences in activity. The enantiomorphic forms of ethyl 4-dimethylamino-2:2-diphenylpentanoate (XXXII) have

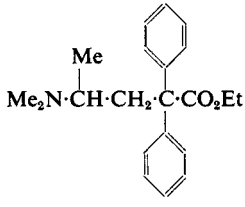


(XXX)

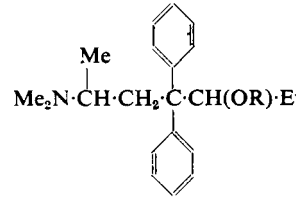


(XXXI)

been prepared from (+)- and (−)-3-dimethylamino-1:1-diphenylbutyl cyanide respectively. Chen[54] reports the (+)-isomer to be 7 times as active as the (−)-form. Reduction of the ketonic group of methadone



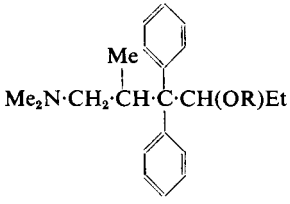
(XXXII)



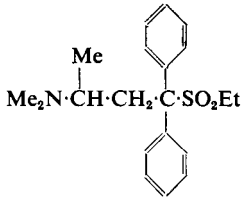
(XXXIII)

to a secondary alcohol introduces a second asymmetric centre. Catalytic hydrogenation, or treatment with lithium aluminium hydride, gives only one of the two possible racemic mixtures, but reduction with sodium and propanol gives both forms (α- and β-methadol, XXXIII, R = H). Reduction of (+)- and (−)-methadone has made available the four possible optical isomers of methadol. The pharmacology of these isomers has been studied by Eddy *et al.*[55] who found α-(−)- and β-(−)-methadol to be from 7 to 8 times as active as their respective enantiomorphs. The corresponding *O*-acetyl derivatives (XXXIII, R = Ac) show greater analgesic properties than the parent compounds. α-(+)- and

β-(−)-acetylmethadol are respectively 6 and 10 times as active as their enantiomorphs[55]. It is significant that, of the four enantiomorphic pairs, the most active member is derived, in three cases, from analgesically active (−)-methadone. The enantiomorphic forms of the *iso*methadols (XXXIV, R = H), and their acetyl derivatives (XXXIV, R = Ac), also exhibit differences in analgesic properties[56]. It



(XXXIV)

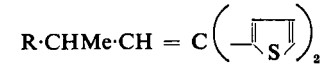


(XXXV)

is to be noted that, in this series, the three compounds showing significant analgesic activity, namely, β-(+)-*iso*methadol, β-(−)- and α-(+)-acetyl*iso*methadol, are all derived from (−)-*iso*methadone. Thus, in both the methadol and *iso*methadol series, the more analgesically active isomers are derived, with the exception of α-(−)-methadol, from the more analgesically active enantiomorph of the parent compound. Replacement of the −COEt group of methadone by the ethyl sulphone group (−SO_2Et) gives a compound of comparable analgesic activity[57]. The sulphone (XXXV) has been resolved and the (−)- found to be 20 times as active as the (+)-isomer[58].

(*f*) *The Dithienylbutenylamines.* This most recently developed group of analgesics, comparable in activity with methadone, also provides examples of enantiomorphic pairs which differ in their analgesic activity. (+)-3-Dimethylamino (XXXVI, R = NMe_2), (+)-3-diethylamino (XXXVI, R = NEt_2) and (+)-3-pyrrolidino-1 : 1-di-(2′-thienyl)-but-1-enes have been shown to be more active than their corresponding (−)-antimers[19,59].

The importance of spatial configuration in analgesics is most clearly demonstrated in those compounds possessing one asymmetric carbon atom. In order to obtain evidence of the stereochemical requirements of analgesics, the present authors carried out configurational studies among a group of analgesics of this type[60]. (−)-(XXX), (−)-(XXXV), (+)-(XXXVI, R = NMe_2) and (+)-(XXXVI, R = NEt_2), each substance representing the more analgesically active member of an enantiomorphic pair, were correlated by unequivocal stereospecific routes and shown to possess identical configurations related to D-(−)-alanine. It was shown[19] that these relationships, together with the evidence of analgesic antagonists, provide support for the hypothesis that a substance must possess an overall optimum spatial arrangement of groups in order to show analgesic activity. Evidence was presented for the probability of the activity exhibited by analgesics and their antagonists being due to

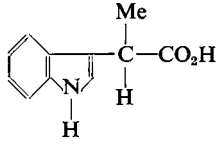


(XXXVI)

association with a specific receptor site, and the differences in analgesic activity between members of enantiomorphic pairs explained in terms of this hypothesis. Highly active analgesics were shown to possess structures which allow of their association with a proposed "analgesic receptor surface," the essential features of which were described.

Plant Growth Substances

It has long been established that substances exist in the tips of growing seedlings which cause the cells to elongate with consequent increase in size of the plant. In 1934, Kögl[61] isolated a substance with pronounced plant growth regulating activity identified as 3-indoleacetic acid. Stereochemical aspects soon received attention when it was found that (+)-α-(3-indole)-propionic acid (XXXVII) was about 30 times more active than the (−)-isomer[62]. Koepfli, Thimann and Went[63], as a result of structure-activity studies, proposed the following generalisations as requirements for an active molecule.



(XXXVII)

(1) a ring system containing at least one double bond,

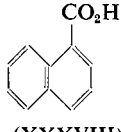
(2) a side chain possessing a $-CO_2H$ group (or a group easily converted into a $-CO_2H$ group),

(3) at least one carbon atom between the ring and the $-CO_2H$ group,

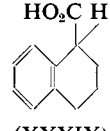
(4) a particular spatial relationship between the ring system and the $-CO_2H$ group.

The latter requirement, of special interest from a stereochemical viewpoint, is exemplified by the variation in activity found among certain *cis-trans* isomeric pairs. Thus, *cis*-cinnamic acid is active, while the *trans*-isomer is inactive[64]. *cis*-2-Phenyl-*cyclo*propane-1-carboxylic acid and *cis*- 1:2:3:4-tetrahydronaphthylidene-1-acetic acid are plant growth stimulating substances, the *trans* isomers in both cases being without activity[65,66].

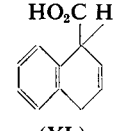
In the examples cited, molecular models reveal the ring and carboxylic acid groups to be almost planar in the *trans* and non-planar in the *cis* isomer. Veldstra[66,67] considered that the *cis* forms owed their biological activity to this factor and explained the increase in growth regulating activity which results on hydrogenating α-naphthoic acid (XXXVIII) to the 1:2:3:4-tetrahydro analogue (XXXIX) in the same terms[68]. This



(XXXVIII)



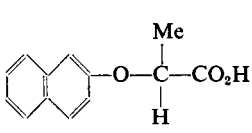
(XXXIX)



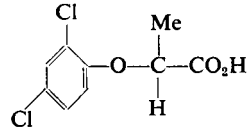
(XL)

hypothesis became inadequate, however, when it was found that the activity of the racemic compound (XXXIX) resided almost entirely in the (+)-isomer[68]. Likewise, the report of Mitsui[69], that the (+)-isomer of 1:4:-dihydro-1-naphthoic acid (XL) is more active than its enantiomorph, was unaccountable on these grounds. Wain and his

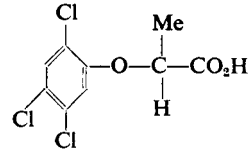
colleagues[70,71] have shown the (+)-isomers of α-(2-naphthoxy)-(XLI), α-(2:4-dichlorophenoxy)-(XLII) and α-(2:4:5-trichlorophenoxy) (XLIII)-propionic acids to be highly active growth substances, while the



(XLI)

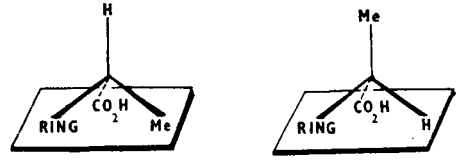


(XLII)



(XLIII)

corresponding (−)-isomers possess little or no activity. Wain[72] explained these results in terms of differences in the "fit" of enantiomorphs at a receptor site. The workers at Wye College have demonstrated the essential nature of the α-hydrogen atom in substances possessing growth stimulating activity, and Wain pointed out that an α-hydrogen atom, an unsaturated ring system, and a carboxyl group, make up three essential structural requirements for compounds of this type. These features must be orientated in a specific configuration in order that the molecule may "fit" the receptor surface. In aryloxycarboxylic acids, all three groupings are attached to an asymmetric centre and it follows that only one enantiomorph will be able to present the three groups in the correct relative positions to the surface and so initiate a growth response. (See Fig. 8: (a) represents the biologically active and (b) the inactive isomer).



(+)-isomer (a) (−)-isomer (b)

FIG. 8

Support for this theory has been provided both by the establishment of configurational identity among the more biologically active members of enantiomorphic pairs, and also by demonstration of the antagonism of certain optically active plant growth stimulating substances by their corresponding inactive enantiomorphs. Much of the evidence has been provided by Fredga and Matell[73,74] in the course of an intensive study of acids of type (XLIV), made with a view to establishing the stereochemical specificity of optically active plant growth substances. Many variants of the basic formula were prepared, and the configurations of the enantiomorphs determined, mainly by the method of quasi-racemates,

$$Ar—X—CHR—CO_2H$$

(XLIV)

Ar = Aromatic Moiety, R = alkyl group, X = O, S, NH or CH_2

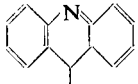
and related to optically active alanine and lactic acid. Thus (+)-α-phenoxy-, (+)-α-(4-chlorophenoxy)-, (+)-α-(2:4-dichlorophenoxy)-, (+)-α-(3:4-dichlorophenoxy)-, (+)-α-(2:4:5-trichlorophenoxy)-, (+)-α-

(2-methyl-4-chlorophenoxy)-, (−)-α-(1-naphthoxy)-, (+)-α-(2-naphthoxy)- and α-(1-chloro-2-naphthoxy)-propionic acids, (+)-α-phenoxy, (+)-α-(2:4-dichlorophenoxy)-, (−)-α-(1-naphthoxy)- and (+)-α-(2-naphthoxy)-butyric acids and (+)-α-(2-naphthoxy)-*n*-caproic acid, each substance being the more biologically active member of an antimeric pair, are all related to D-alanine. Although the configurations of the more active isomers of α-(1-naphthylmethyl)-, α-(2-naphthylmethyl)- and α-(2-naphthylsulphide)-propionic acids were not determined, the Swedish workers considered it very probable that these isomers also were related to D-alanine. Correlation between biological activity and configuration has similarly been reported by Mitsui[75] for (XL) and (XXXIX), and by Veldstra[68] for the latter acid and α-allylphenylacetic acid.

Evidence from antagonism studies has been obtained by Wightman[71] who examined (+)-α-(2-naphthoxy)-, (+)-(2:4-dichlorophenoxy)- and (+)-α-(2:4:5-trichlorophenoxy)-propionic acids in the presence of increasing amounts of their corresponding inactive enantiomorphs. In each case, he found that the inactive (−)-isomer could reduce and, with a high molar ratio, even eliminate the activity of the (+)-isomer in this test. Åberg, in the course of biological examination of enantiomorphic α-phenoxypropionic and α-naphthoxypropionic acids, tested their antagonistic action against 2:4-dichlorophenoxyacetic acid and demonstrated the antagonistic properties of (−)-α-(2-methyl-4-chloro-phenoxy)-, (−)-α-(2:4:5-trichlorophenoxy)-, (−)-α-(2-naphthoxy), (−)-α-(1-chloro-2-naphthoxy)-, (+)-α-(1-naphthylmethyl)-, (−)-α-(2-naphthylmethyl) and (−)-α-(2-naphthylsulphide) propionic acids, (−)-α-(2-naphthoxy)-*n*-butyric acid and (−)-α-(2-naphthoxy)-*n*-caproic acid[73]. Steward[76] has found a synergistic action to exist between the cocoanut-milk growth factor and plant growth stimulating substances. He showed that (+)-α-(2:4:5-trichlorophenoxy)- and (+)-α-(2-naphthoxy) propionic acids were highly active in this respect, whereas both of the corresponding (−)-isomers were completely inactive[72]. Furthermore, the inactive (−)-isomers antagonised the synergistic action of their enantiomorphs[72].

Antibacterials

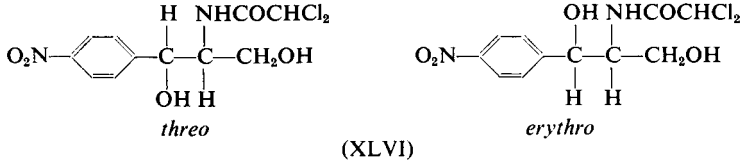
Many antibiotics have been shown to prevent the growth and reproduction of micro-organisms by interference with cell synthesis. It is possible that this action might be achieved by the introduction into the cells of "unnatural" D-amino-acids, such as are present in certain antibiotic polypeptides (e.g., gramicidin-D, tyrocidine and gramicidine-S). Support for this contention is provided by the antibiotic activity of synthetic penicillin derived from D-penicillamine (ββ-dimethylcysteine) and the inactivity of material obtained from the corresponding L-isomer[77]. Linnell and Smith[78] resolved compound (XLV), comprising acridine and an amino-acid moiety, and found the (+)-isomer to be twice as effective as the (−)-isomer in inhibiting the growth of *Staph. aureus* and *Strept. pyogenes* (configurations were not elucidated). Work[79], however, has



(XLV)

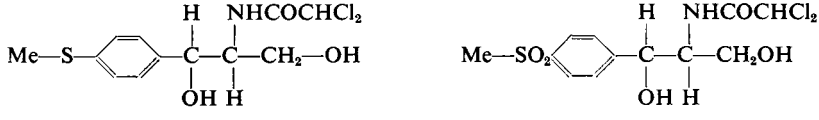
pointed out that the lack of evidence of the inhibitory effects of D-amino-acids upon protein synthesis, renders the above view of the role of these acids in antibiotic action unlikely. He considers it more probable that the configurations of the component amino-acids are of importance in determining "fit" of the antibiotic molecule at a specific receptor surface within an enzyme system.

Conclusive evidence of the stereospecificity of antibiotics has been provided by study of chloramphenicol and related substances. The four optical isomers of 1-*p*-nitrophenyl-2-dichloroacetamido-1 : 3-propanediol



(XLVI)

(XLVI) have been obtained, and their configurations related to norephedrine and ψ-norephedrine[80,81]. While the D-(−)-*threo*-isomer (chloramphenicol) is a potent antibacterial agent, the L-(+)-*threo*, D-(−)-*erythro* and L-(+)-*erythro* isomers possess negligible activity[82]. Furthermore, the antibacterial activities of the racemic compound (XLVII), and its sulphone analogue (XLVIII), have been shown to



(XLVII) (XLVIII)

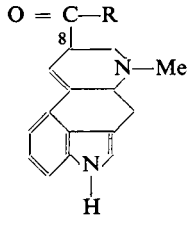
reside, in both cases, in the (+)-isomers, designated D-*threo* on the basis of rotational analogies with chloramphenicol[83,84].

Hahn, Wisseman and Hopps[85], in a study of the mode of action of chloramphenicol, pointed out the antipodal relationship between the D-(−)-*threo* isomer and the L-polypeptides whose formation is inhibited. If this relationship be significant, the D-(−)-*threo* isomer should have no influence on D-polypeptide synthesis; the latter should be inhibited, however, by the L-(+)-*erythro* analogue of chloramphenicol in virtue of an analogous but converse relationship. Hahn *et al.*[85] confirmed these conclusions by experiment. Formation of D-(−)-glutamyl polypeptide by *B. subtilis* was inhibited specifically by the L-(+)-*erythro* analogue of chloramphenicol but not by the antibiotic itself. The same stereoisomer had little effect on the growth of the test organism while chloramphenicol completely suppressed its growth at a low concentration. The D-(−)-*erythro* and L-(+)-*threo* isomers had no effect on either growth or polypeptide formation.

Miscellaneous

Physiologically active ergot alkaloids are all derivatives of lysergic acid (XLIX, R = OH); they occur in association with inactive stereoisomers, derived from *iso*lysergic acid. The parent acids are epimeric

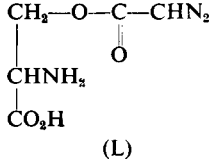
and differ only in the $C_{(8)}$-configuration, the latter, therefore, having a critical influence upon physiological activity. The configuration of the non-lysergic acid moiety (XLIX, R) does not appear to play such an important role in the determination of activity. Stoll[86] found ergometrine (XLIX, R = NH·CH(Me)·CH_2OH) derived from L-alaninol and material obtained from D-alaninol to possess equal potencies. However, when (+)-lysergic acid is combined with norephedrine, the compound with the natural (−)-form is found to be 20 times more active than that with the (+)-isomer.



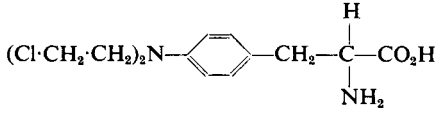
(XLIX)

Reports on the relative physiological activities of (−)- and (+)-thyroxine, although somewhat conflicting, establish the (−)-isomer to be the more active in a wide variety of tests., e.g., oxygen consumption and weight curves of rats[87]; metamorphosis of tadpoles[87,88]; reduction of hyperplastic thyroids[88]; prevention of pituitary basophil changes[89]. In contrast, Salter *et al.*[90] found the so-called calorigenic activities of (−)- and (+)-thyroxine in persons suffering from myxœdema to be nearly equal.

The amino-acid derivatives, *O*-diazoacetylserine (L) (L-isomer is azaserine, isolated from a *Streptomyces*) and *p*-di-(2-chloroethyl)amino-



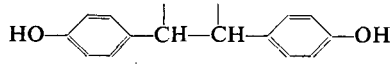
(L)



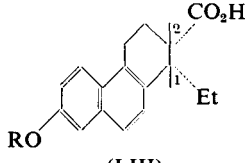
(LI)

phenylalanine (LI) possess antitumour activity; maximum activity has been shown to reside, in both cases, in the L-isomer[91,92].

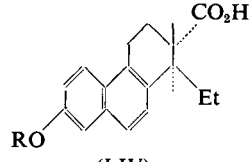
$\gamma\delta$-Bis-(4-hydroxyphenyl)-*n*-hexane exists in two optically inactive forms; the *meso* compounds (LII, hexœstrol), a potent œstrogen, and the much less active racemic mixture (*iso*hexœstrol). The latter has been resolved and the (+)-found to be 10 times more potent than the (−)-isomer[93]. Alkali fusion of (+)-equilenin gives rise to (−)-α- and (+)-β-bisdehydrodoisynolic acids. High œstrogenic activity depends on



(LII)



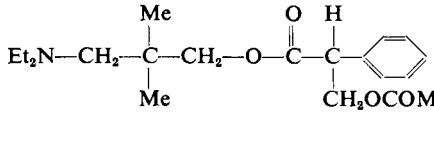
(LIII)



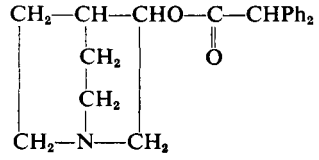
(LIV)

cis-orientation of $C_{(1)}$—Et/$C_{(2)}$—CO_2H, (LIII, R = H) possessing high activity while (LIV, R = H) is inactive[94]. The (−)-α-7-methylether

(LIII, R = Me) is equally active, the corresponding (+)-isomers of both α-compounds possessing virtually no œstrogenic properties[95].



(LV)



(LVI)

The antimeric forms of the synthetic spasmolytics (LV) and (LVI) show differences in activity; in both cases the (−)-isomer possesses the greater action[96,97]. Blood pressure depressants show similar differences; (+)-adenocarpine produces a greater fall in blood pressure in the cat than the (−)-form[98]; the depressant action of (±)-carnosine (formed from histidine and β-alanine) is due entirely to the L-isomer[99]. D-Cysteine is not as effective as L-cysteine in preventing the leucopenia and neutropenia induced by nitrogen mustard[100]. The nicotinolytic activity of (−)-Parsidol [N-(2-diethylaminopropyl)-phenothiazine] is twice that of the corresponding (+)-base[101].

The above examples of stereochemical specificity in biological action serve to indicate the importance of stereochemical investigations of biologically active structurally specific compounds. Although a particular biological response to a compound may be influenced by many factors, studies of the actions of enantiomorphs in which so many properties are identical, enables some of the fine structure of receptor surfaces to be delineated when similar configurations can be established for the more active members of enantiomorphic pairs exhibiting a particular biological action. The use of geometrical isomers which are dissymmetric also offers an approach to the elucidation of both the distances, and the orientation of specific receptor areas of the receptor site, if the differences in the physical properties of the geometrical isomers are not great. The combination of such studies with the investigation of the antagonistic action of stereoisomers, and the change in the biological effect upon alterations of the groups necessary for "combination" at the receptor, has great potentialities in the search to unravel the complexities of the mechanisms by which biological responses are mediated.

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