RELATIONSHIPS BETWEEN THE CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY OF CONVULSANTS[1]

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Introduction

A large part of classical pharmacology consists of experimental determinations of the relationship between chemical structure and biological activity. This study of structure-activity relationships (SAR) has proceeded by the random syntheses of a large number of chemical relatives of known agonists or antagonists of a particular transmitter or hormone and testing their activity on the appropriate isolated tissues. Another approach is to synthesize some quite novel chemical structure and then test it on a wide variety of biological systems in the hope that this trial-and-error approach has on this occasion achieved a hit. The "lock-and-key" model of transmitter-receptor and hormone-receptor interactions introduced by Fischer presupposes that a close complementarity exists between the molecular structure of the active drug and the molecular structure of the receptor. However, as nothing was known about the molecular structure of receptors, the SAR data could not be interpreted except in the vaguest and most general terms. It is clear that, if only we knew the precise molecular structure of a particular receptor, it would be possible to explain just why certain drugs were agonists, and others antagonists, at this receptor and also to design new drugs on a rational basis (Martin-Smith 1). It is still true that we have no certain knowledge about the structure of any receptor, owing to the formidable technical difficulties involved in isolating the receptor protein and determining its amino acid sequence and tertiary conformation. However, it has proven possible to deduce a general theory of the molecular structure of protein-based receptors based on a search for complementarity between drugs known to act at these receptors and the range of possibilities of protein structures that could be

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involved (Smythies 2). This general theory has been successful in explaining the bulk of the SAR data and a detailed specification has been made in the case of the following receptors: acetylcholine (neuromuscular junction, ganglionic, and muscarinic); GABA, glutamate, and glycine; α- and β-adrenergic and dopamine; and various prostaglandins. A partial specification has been made for the serotonin receptor. The same technique has been used to specify the molecular structure of the sodium channel with explanations for the mechanism of action of tetrodotoxin, saxitoxin, batrachotoxin, aconitine, veratridine, and the local anesthetics of the procaine class (Smythies 2). It has also been applied to presynaptic storage and release mechanisms for acetylcholine and adrenaline, carrying explanations for the mechanism of action of morphine, scorpion neurotoxin, and reserpine (Smythies 2, Smythies et al 3). The general hypothesis has given rise to a number of specific predictions which are currently under experimental test in a number of centers. This review will concentrate on an aspect of the general theory most amenable to such crucial tests, namely the GABA and glycine receptors.

Brief Statement of the General Theory

My hypothesis is based on a combination of the Kusnetsov & Ghokov (4), Gill (5), and Barlow (6) hypotheses. The former suggested that the most likely form of the receptor protein was parallel β chains cross-linked by their opposing amino acids by hydrogen or ionic bonding. Gill (5) suggested that the most likely mode of action of agonists like acetylcholine and the amino acid transmitters with their strongly charged groups was to disrupt an ionic link between two amino acids bearing opposite charges. Barlow (6) suggested that the ACh receptor in the neuromuscular junction contained a grid of anionic sites some 14 Å apart. Investigations using Corey–Pauling–Kaltun (CPK) molecular models suggested that a Kusnetsov–Ghokov–Barlow–Gill grid could be constructed if the receptor was based on four rungs of such a ladder composed of ionically bonded Arg and Glu moieties (Figure 1). The SAR data further indicated that such a flat, two-dimensional grid could be converted into a three-dimensional receptor cup by the addition of two further segments of protein chain (secondary chains) to the two (primary) chains of the grid. Each secondary chain forms in part a formal β-pleated sheet with the primary chain and in part runs over one outer rung of the ladder. The receptors for different transmitters may now be specified by the Arg-Glu vs Glu-Arg sequence of the primary chains, and by differences in the amino acid sequence of the secondary chains [there are three main types of secondary chain: F, E, and DN (see below)] and by the direction the protein chains run, as detailed in my book (2).

TESTING THE HYPOTHESIS In the case of certain receptors such as the cholinergic neuromuscular junction or muscarinic receptor a wide variety of compounds are known that block them. CPK molecular models of any of these may be placed in a CPK model of the postulated receptor and derivatives can be designed that should either be inactive or more active depending on the strategic placement of additional lipophilic, hydrogen bonding, or ionic groups. Work along these lines is proceeding in a number of centers. However, in the case of other receptors much less is known

in the SAR field and so a new strategy becomes possible as outlined in the next section.

Convulsant Drugs and the GABA and Glycine Receptors

Although there are a large number of convulsant poisons known, the mechanism of action of only a few of these has been worked out—e.g. strychnine, which blocks the glycine receptor, and picrotoxin and bicuculline, which block the GABA receptor. Of course not all convulsants act by blocking one or other of these receptors. Some, such as allylglycine,[1] block the enzyme glutamate decarboxylase and thus lower brain GABA levels. Others act by other mechanisms. However, a study of the molecular structure of a number of known convulsants has enabled me to predict with confidence which will have anti-GABA and which will have antiglycine properties.

The specification of the GABA receptor was based on the molecular structure of GABA, picrotoxin (Figure 5b), and bicuculline (Figure 5a) and that of the glycine receptor on glycine and strychnine. The primary structure of the GABA receptor is Glu-Arg; Arg-Glu; Glu-Arg; Glu-Arg; and the secondary chains have the sequence (*right*) *-x-His-x-Val* (or Ile)-Pro-Gly-x-Gly- (an E chain) and (*left*) -x-Gly-x-Gly-Pro-*x-Ile-x-Asp-x* (an F chain) with the italic portion forming the β-pleated sheet structure with the underlying primary chain in each case (Figure 2). The glycine receptor has only 3 rungs instead of 4: primary structure Arg-Glu; Glu-Arg; Glu-Arg and secondary chains (both DN) have the sequences -Gly-x-Gly-Pro-x-*Asp*-x and -Gly-x-Gly-Pro-x-*His*-x.

The GABA Receptor

GABA binds in its model receptor with its carboxyl group caught between three protons (2 from Arg of rung 3 and 1 from His), its amino group binds to the Glu of rung 2 (repelling Arg of rung 2), and its α-methylene group binds lipophilically to the adjacent Ile.

The molecule of bicuculline forms a lid over the receptor cup binding as diagrammed in Figure 3 and picrotoxin binds as suggested in Figure 4. Its square upper section slots into the rectangular "inside" of the F secondary chain with extensive lipophilic interactions and a hydrogen bond from OH to His. The two carboxyl oxygens now jut down with the correct locations and bond angles to receive hydrogen bonds from the two spare protons on the two Args (of rungs 2 and 3) in the floor of the receptor.

Based on this molecular structure it has been possible to predict that the following convulsant poisons act by competitive inhibition (blockade) of the GABA receptor. In each case an outline of the molecular complementarity between drug and receptor is given. A perusal of Figures 5–9 will emphasize the remarkable range of chemical structures involved. Nevertheless the molecular models indicate that they are all complementary to the simple protein structure specified.

[1]The action of allylglycine in inhibiting GAD (32) has been challenged by Roper (33).

1. Cicutoxin (Figure 5e). This remarkable molecule may be considered as a dense π cloud bent in the middle through some 40° with a lipophilic "tail" at each end, to each of which is attached an hydroxyl group. Figure 6 indicates how this is related to the model receptor. The π cloud contributed to by the ethylene and acetylene π electrons crosses the 2 spare protons of both arginines in the floor of the receptor (thus forming 4 hydrogen bonds: for this the 40° bend is required). One hydroxyl binds to His (N:) and the other to Asp (O:) and both propylene chains make extensive lipophilic contacts. The related molecules of oenanthotoxin (Figure 5f) and cunaniol make similar contacts.

2. Tetramethylenedisulphotetramine (Figure 7a). This small compact molecule is totally different in form from cicutoxin yet it is also most closely related to the model receptor. It sits in the middle (Figure 8) caught between His, Asp, Val, Ile, and the two Args with 3 hydrogen bonds, 1 ionic bond, and 3 lipophilic contacts with all these as shown in the figure.

3. Kopsine (Figure 7b) is of particular interest, as the line diagram suggests it has a resemblance to strychnine and therefore might be expected to block glycine receptors. However, the molecular models indicate it is too large to fit the smaller glycine receptor and is adapted to fit the larger GABA receptor instead. The ring carbonyl O receives a hydrogen bond from the Arg NH in the floor of the receptor, the protonated amino group binds to Asp, and the other carbonyl O receives a hydrogen bond from His. The hydroxyl forms an internal hydrogen bond to the methoxy O so as to orient the adjacent carbonyl O correctly. There are also extensive lipophilic contacts. The molecule thus combines the attributes of both picrotoxin and bicuculline.

4. Figure 7c shows the formula of the synthetic convulsant dicetol and also predicts which out of the 16 possible stereoisomers will be most active. Each carbonyl O binds to Arg NHs in the floor of the receptor, one hydroxyl binds to His and one to Asp, and there are extensive lipophilic contacts to Ile and Val on each side.

5. Two bicuculline-like structures are the synthetic compounds 3-methyl-7-methoxy-8-(dimethylaminomethyl)-flavone (Figure 7d) and the equivalent chromone (Figure 7e).

6. Yet another way of achieving the same effect is illustrated by dregamine (Figure 7f) based on an indole ring.

7. The skin of European fire and Alpine salamanders is chemically most interesting as it contains two convulsant poisons: one, samandarine (Figure 9a), is complementary to the GABA model receptor and the other, cycloneosamandione (Figure 9b), is complementary to the glycine receptor.

8. The latest convulsant alkaloid shown to be a GABA antagonist (Curtis 7) is Shikimin (Figure 5d). The molecular model shows it to be a homograph of picrotoxin but with more extensive binding to elements in the receptor. Each carbonyl O receives a hydrogen bond from an Arg NH underneath. The two hydroxyls on the five-carbon ring can now both bind electrostatically to the same O of Asp, and the hydroxyl on the six-carbon ring forms an electrostatic link to the O: of Glu of rung 2 and the fourth hydroxyl binds to His N:. The hydrocarbon ring system plus

one attached methyl group (that on the six-carbon ring) form lipophilic bonds with Val and Ile (Figure 4b).

9. The convulsant compound benzyl penicillin (Figure 5g) cannot be made by the CPK models available, owing to its four-carbon ring. However, an approximation to this made out of plasticine indicates that it has the essential square form plus two carbonyl pseudopods to be a picrotoxin-like GABA antagonist.

10. A GABA antagonist bascd on the steroid ring may be represented by 3β-acetoxy-5α-hydroxy-6β-morpholino-5α-pregnan-20-one (Figure 9c).

The Glycine Receptor

Glycine (Figure 12a) binds to its model receptor with its amino group caught between Asp and Glu, its carboxyl group between His and Arg, and there is a lipophilic contact between its single methylene group and an adjacent Gly moiety in the receptor protein in addition (Figure 10). The squat squarish molecule of strychnine fills the receptor cup almost completely with an ionic bond from its protonated amino N to Asp and a hydrogen bond from His to its carbonyl O, as indicated in Figure 11a. The molecules of lordanosine (Figure 12b), thebaine (Figure 12c), and dendrobine (Figure 12d), which are also known to act by blocking the glycine receptor, are all complementary to this model receptor in different ways (see Figure 11b,c).

Antiglycine Compounds

The model predicts that the following compounds owe their convulsant properties to blockade of the glycine receptor.

1. Akuammine (Figure 13a). This is not surprising since it is a close chemical relative of strychnine; however, superficially it also resembles kopsine (Figure 7b), which as we have seen fits my GABA receptor and not the glycine receptor model.

2. Securenine (Figure 13b). This resembles picrotoxin adapted to fit the glycine receptor with a single pseudopod (the carbonyl O), making contact with the Arg NH in the floor of the receptor cup and the NH group binding to Asp.

3. Dioscorine (Figure 13c). It achieves a similar result using a different chemical structure.

4. Calycanthine (Figure 13d). This fits the receptor cup quite differently. The molecule has a right-angled bracket shape (as does thebaine). One benzene ring intercalates between the rungs of the primary "ladder" structure and the other forms a "lid." One amino N binds to Asp ionically and the other forms a hydrogen bond to His.

5. 5,7-Diphenyl-1,3-diazadamantan-6-ol (Figure 13e). In the case of this compact molecule both benzene rings intercalate into the inter-rung space: one between rungs 1 and 2 and the other between rungs 2 and 3. One amino group binds to Asp and the hydroxyl to His.

New Compounds

A perusal of recent copies of *Chemical Abstracts* reveals a wealth of activity in the chemical identification of new alkaloids. In fact this process has outstripped the

pharmacological estimate of what biological action, if any, these new compounds might have. A comparison of molecular models of a wide range of these new compounds suggests that a small number may have convulsant properties, to wit:

PREDICTED ANTI-GABA COMPOUNDS

1. Secodaphniphylline (Figure 14a). This achieves a remarkably close fit to the entire cavity of the model receptor cup with bonds to Asp (NH) and His (carbonyl O) as well.

2. Taspine (Figure 14b) and Thaliglucinone (Figure 14c). These resemble the convulsant chromone listed above (Figure 7e).

PREDICTED ANTI-GLYCINE COMPOUNDS

1. 4-Hydroxydendroxine (Figure 14c).

2. Elegantine (Figure 14d). This fills the glycine receptor cup much as secodaphniphylline fills the GABA receptor cup.

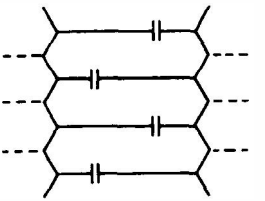


Figure 1 An Arg-Glu grid. The short apposed amino acid is Glu and the long one is Arg.

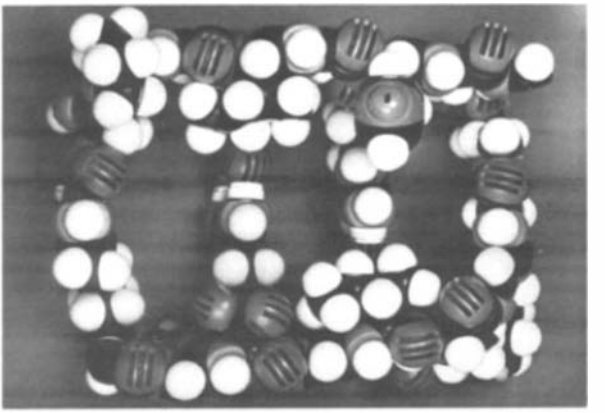


Figure 2 A CPK model of the postulated GABA receptor.

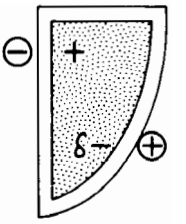


Figure 3 A diagram of how the trapezoid-shaped molecule of bicuculline fills the trapezoid-shaped GABA receptor cup with two complementary electrostatic bonds to His (+) and Asp (-) in the receptor protein.

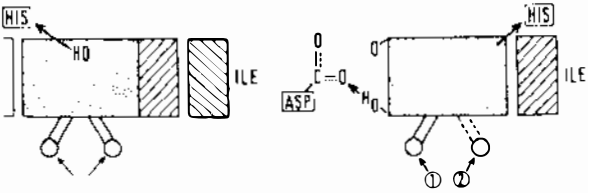


Figure 4 Diagram of suggested mode of binding of *a.* picrotoxin and *b.* shikimin in the GABA receptor. The arrows indicate correct hydrogen bonds from Arg NHs to Carbonyl Os.

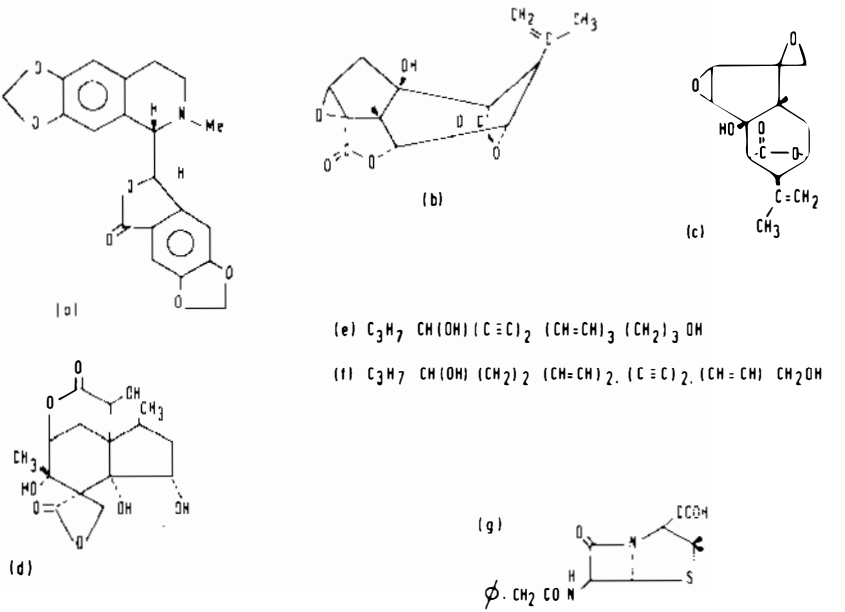


Figure 5 *a.* Bicuculline; *b.* picrotoxin; *c.* coryamertin; *d.* shikimin; *e.* cicutoxin; *f.* oenanthotoxin; *g.* benzyl penicillin.

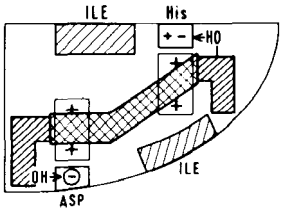


Figure 6 Diagram of interaction between cicutoxin and the model GABA receptor.

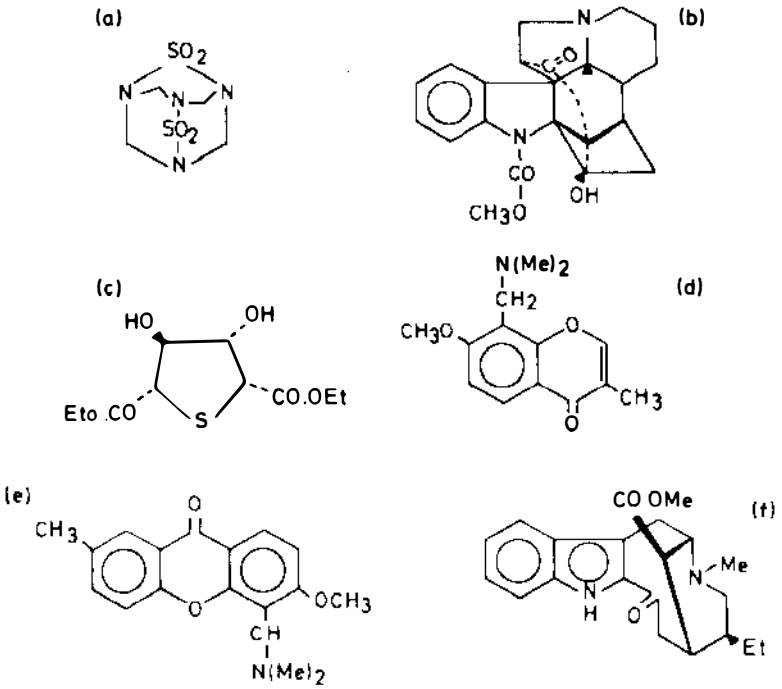
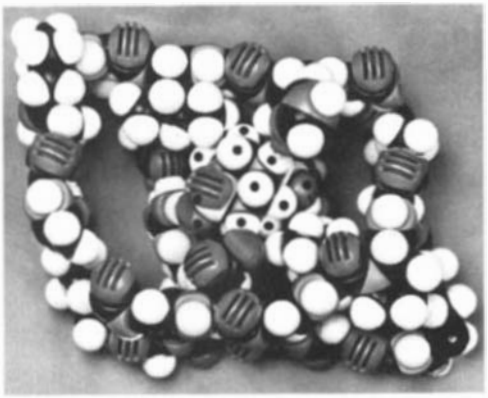


Figure 7 *a.* Tetramethylenedisulphotetramine; *b.* kopsine; *c.* dicetol; *d.* 3-methyl-7-methoxy-8 (dimethylaminomethyl)-flavone; *e.* the equivalent chromone; *f.* dregamine.



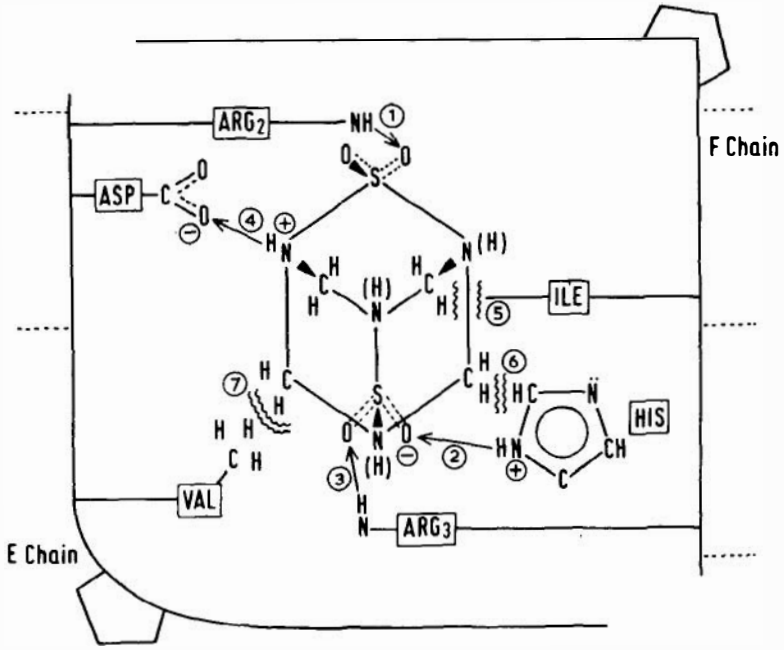


Figure 8 *a.* CPK model (*top*) and *b.* diagram of tetramethylenedisulfotetramine bound in the model receptor (*bottom*).

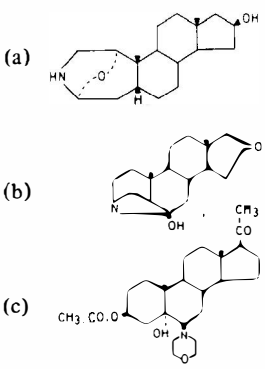


Figure 9 (*left*) *a.* samandarine; *b.* cycloneosamandione; *c.* 3β-acetoxy-5α-hydroxy-6β-morpholino-5α-pregnan-20-one.

Figure 10 (*right*) CPK model of glycine bound in its model receptor.

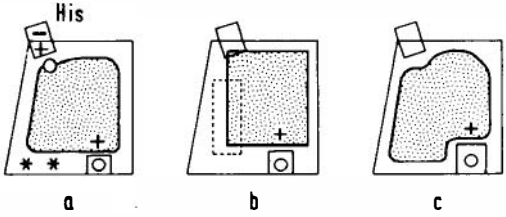


Figure 11 Diagram of how various convulsants fill the glycine receptor. *a.* strychnine: the two stars indicate where the two methoxy groups of brucine fit; *b.* lordanosine; *c.* dendrobine: the circle in the square represents Asp.

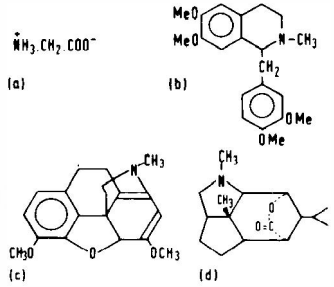


Figure 12 *a.* Glycine; *b.* lordanosine; *c.* thebaine; *d.* dendrobine.

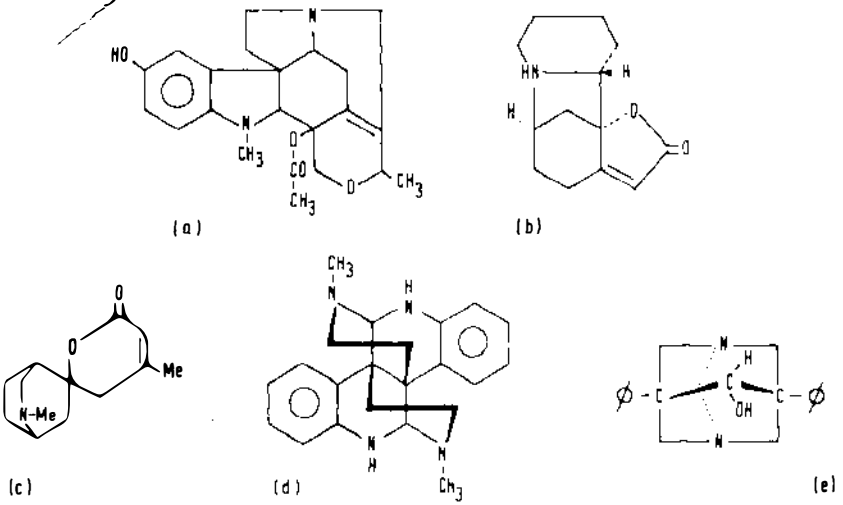


Figure 13 *a.* Akuammine; *b.* securenine; *c.* dioscorine; *d.* calycanthine; *e.* 5,7-diphenyl-1,3-diazadamantan-6-ol.

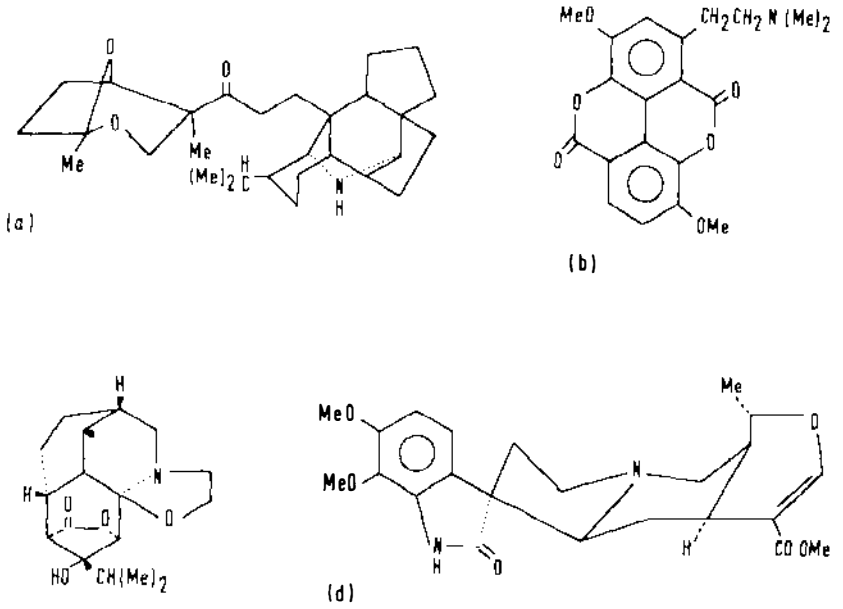


Figure 14 *a.* Secodaphniphylline; *b.* taspine; *c.* 4-hydroxydendroxine; *d.* elegantine.

ACKNOWLEDGMENTS

I am grateful to Professor David Curtis for the benefit of many helpful discussions and to Professor Sir John Eccles, FRS, for the interest he has kindly shown in these ideas.

APPENDIX Further information about the compounds cited.

1. Cicutoxin. Source: *Cicuta virosa* L. *Umbelliferae* (Water Hemlock) *Ref.* (8)
2. Oenanthotoxin. Source: *Oenanthe crocata Umbelliferae* (Hemlock Water Dropwort) (W. Europe) *Ref.* (9)
3. Cunaniol. Source: *Clibadium Sylvestre* (Aubl.) *Baill.* (Brazil) *Ref.* (10)
4. Tetramethylenedisulphotetramine. Source: Synthetic. *Ref.* (11, 12)
5. Kopsine. Source: *Kopsia fructicosa* A.D. *Apocynaceae* (Java) *Ref.* (13, 14)
6. 2,5'-dicarbethoxy-3,4-dihydroxy-thiophane (dicetol). Source: Synthetic. *Ref.* (15, 16)
7. 3-methyl-7-methoxy-8-(dimethylaminomethyl)-flavone (and equivalent chromone). Source: Synthetic. *Ref.* (17, 18)
8. 3β-acetoxy-5α-hydroxy-6β-morpholino-5α-pregnan-20-one. Source: Synthetic. *Ref.* (19)
9. Samandarine and cycloneosamandione. Source: Skin of European Fire and Alpine Salamander. *Ref.* (20)
10. Dregamine. Source: *Voacanga dregei Apocynaceae* and *Tabernae montana* Sp. *Apocynaceae* (Madagascar). *Ref.* (21)
11. Securenine. Source: *Securinega suffructicasa* Rehder, *Euphorbiaccae* (Ussuri region, USSR) *Ref.* (22)
12. Akuammine. Source: *Picralima klaineana,* also *Vinca major* (L) *Ref.* (23)
13. Calycanthine. Source: *Calycanthus floridus* L. *Calycanthaceae* (S.E. United States) *Ref.* (24)
14. Dioscorine. Source: *Dioscorea hispida* Deunst. *Ref.* (25)
15. 5,7-diphenyl-1,3-diazadamantan-6-ol. Source: Synthetic. *Ref.* (26)
16. Secodaphniphylline. Source: *Daphniphyllum macropodum Ref.* (27)
17. Taspine. Source: *Leontice eversmanii.* Bunge, *Berberidaceae Ref.* (28)
18. Thaliglucinone. Source: *Thalictrum rugosum Ref.* (29)
19. 4-hydroxydendroxine. Source: *Dendrobium nobile* (*Orchidaceae*) *Ref.* (30)
20. Elegantine. Source: *Vinca elegantissima* Hort. N. O. *Apocynaceae* (rare herb growing in Nilgiri Hills Assam). *Ref.* (31)

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