ANTICONVULSANT DRUGS BASED ON THE NEUROCHEMISTRY OF SEIZURES.

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Ammonia, considered as one of the possible endogenous neurotoxins associated with the neurochemical lesion in the epileptogenic tissue, was chosen as a working hypothesis for developing a specific therapy. The intriguing structural characteristics of indolin-2,3-dione (isatin) designed to fix it (ammonia) up have been discussed. It was found to possess a potent anticonvulsant action against maximal electro-shock seizures in rats. Whereas its 3-keto moiety was found to be essential for activity, N-alkylation and acylation inactivated the compound. It may probably also prove useful in cardiac arrythmias and in hepatic dysfunction.

The introduction of bromides in 1857 and of phenobarbitone in 1912 led to the understanding that the seizure was an abnormality of neuronal function or activity which could be controled without seriously interfering with other aspects of CN function. It is significant that in a century of anticonvulsant therapy only three groups of drugs possessing some apparent structural similarity, namely, phenobarbitone, dilantin and tridione, have stood the test of time, although none is completely or universally satisfactory (Tower, 1956). The discovery of dilantin in 1938 resulted in a chain of congeners such as phenurone, mysoline, hibicon, milontin, acetazolamide and some acetylenic carbinols. Their differential action (effectiveness in different types of seizures) and their diverse chemical structures could not decipher any common pharmacophore (Gujral *et al*, 1957 a) responsible for their action upon specific receptors or enzyme systems, if any. A few generalisations, such as the presence of a resonant aromatic nucleus (usually phenyl) in the threshold-stabilisers and seizure-limitors like dilantin, were, however, obvious (Tomen, 1955). As the precise mechanism of action of these compounds is still obscure, it was decided to pursue the development of specific therapy on the alternative rational approach based on the nature of the biochemical lesion in the epileptogenic seizures.

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Basis of selection of the drug.—The precise nature of the neurochemical lesion is not well understood at present. However, the abnormal behaviour of the epileptogenic tissue has been considered, amongst other factors, to be a response of normal neurons to an abnormal local environment. Certain conditions, like anoxia, hypoglycemia and alteration in pH and ionic environments, or substances, like acetylcholine, ammonia, phosphocreatine, guanidine-like substances (Murray and Hofmann, 1940; Murray and Scroggins, 1949) or some other hitherto unknown endogenous substance similar to methionine-sulphoximine, which produces in experimental animals a condition closely resembling human epilepsy, have been implicated as possible agents apparently concerned with the seizure activity (Elliott, 1955 ; Tower, 1960 a). All these substances possess a basic center as a common denominator. It is difficult to discern whether this fact is in any way associated with the isoelectric point (pHi ca. 2) of the cytoplasm of the CNS (Tolstoouhov, 1955). The role of acetylcholine and ammonia, however, have been emphasized more than those of the other agents. Out of these ammonia was chosen as a working hypothesis for the present study to develop newer anticonvulsants.

The *in vivo* detoxification of ammonia, or ammonia-like substance, usually involves a fixation process utilising one or more of the following groups: activated $>CO$, CO_2, ω-COOH and ω-NH_2 which, respectively, are involved in transamination, transcarbamylation, amidation and transamidination reactions via α-keto acids using pyridoxal phosphate, carbamyl phosphate, glutamic and aspartic acids and ornithine and GABA type compounds. A non-toxic molecule which could fix up ammonia and could also cross the blood brain-barrier was designed Although it is difficult to design a molecule merely from structural angles to serve a specific purpose in a particular tissue, the structural characteristics of indolin-2,3-dione (isatin) seemed to be ideal to meet most of these requirements.

Activated $>C=O$ *group.*—Indolin 2,3-dione (α-keto-β-γ-phenylenebutyrolactam) readily fixes up ammonia as well as ammonia-like substances, such as amino acids, guanidines and guanidino-acids, hydroxylamines, hydrazines and semicarbazides (Elderfield, 1952a ; Sumpter and Miller 1954a). The convulsant hydrazides, which have been considered to act by causing vitamin B_6 deficiency (Tower, 1960b ; Roberts and Baxter, 1960a) could also be fixed up. Due to its ability to participate in Strecker reaction, indolin-2,3 dione also serves as a synthetic enzyme (a dehydrogenase) by utilizing, like pyridoxal phosphate, its activated keto group for dehydrogenation (Elderfield, 1952b ; Sumpter and Miller, 1954b), which is in contrast to the isoalloxazine diene-system utilized by the riboflavine-dependent L- and D-amino acid oxidases.

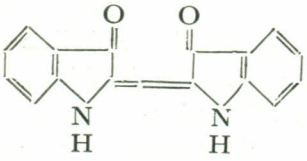
Its lactam nature could facilitate its crossing the blood-brain barrier. Besides, any *in vivo* base-catalysed ring opening to isatinic acid could provide free carboxyl and amino groups, both of which are further capable of fixing up ammonia. The amino group, by carbamylation (cf. ornithine), forms ureide which can even further cyclise to a stable compound.

Resemblance to phenylene-GABA.—The role of GABA in brain metabolism and its (or some of its derivatives ?) possible association with the inhibitory transmitter (Roberts and Baxter, 1960b) has focussed attention on drugs capable of raising its level in the brain. Dilantin has been shown to raise the brain GABA level (Vernadakis and Woodbury, 1960). Due to their inability to cross the blood-brain barrier, exogenous administration of GABA or its precursor, glutamic acid, does not raise its brain level. On the other hand, glutamine which can cross it does not raise its level. Hydroxylamine, which has been reported to raise the brain GABA level (Baxter and Roberts, 1960 ; Eidelberg *et al.*, 1960) is too toxic for any clinical use. Our search was therefore directed to compounds which, instead of being "GABA-genic", could possibly be "GABA-mimetic". In this connection the intriguing resemblance of isatinc acid, α-keto-β-γ-phenylene-GABA, obtainable from isatin under alkaline conditions, seemed to be of interest. The phenyl group of the common anticonvulsants, besides facilitating their penetration of the blood-brain barrier (Tower, 1960c), also confers on them a specific anti-grand mal action as in dilantin, phenobarbitone, mysoline, milontin, epidon, the phenyl analogue of the anti-petit mal, tridione (Gujral *el al.*, 1957a), and 3-phenyl-3-α-pyridyl-2-oxo-pyrrolidine. 2-Pyrrolidinone, GABA-lactam, itself is inactive though its 5-(ω-benzyl)-carboxamide is active as anticonvulsant (Angier, 1953). A phenylene group, as in indolin-2,3-dione, may also be considered to be effective, as evidenced by the activity of 2-alkyl-3-aryl-quinazol-4-ones (Sareen *et al.*, 1959 ; Bianchi and David, 1960; Jackman *et al.*, 1960).

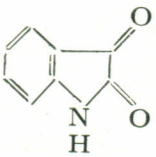
Relation to 5-HT.—Dilantin and several other anticonvulsant drugs have been shown to raise the brain 5-hydroxy-tryptamine (5-HT) level (Bonnycastle *et al.*, 1957). Its precursor, 5-hydroxytryptophane, (5-HTP), and some monoamine-oxidase inhibitors are also known to raise its brain level. Several monoamine-oxidase inhibitors have also been shown to possess anticonvulsant activity (Prockop *et al.*, 1959). Although indolin-2,3-dione has not so far been shown to be a 5-HT protagonist or to raise its brain level, it is an indole derivative entirely different from those which have so far been shown to be 5-HT antagonists[1] (Gaddum *et al.*, 1955).

1. Indolin-2,3-dione is only a non-specific antagonist of 5-HT on the rat uterus or guinea-pig ileum (Gaddum *et al.*, 1955).

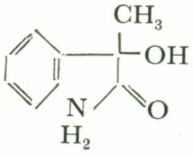
Resemblance to other anticonvulsants.—Indolin-2,3-dione is the oxidation product of indigo which, it is interesting to note, was one of the drugs recommended as anticonvulsant, coincident with the advent of bromides, in the last century (Lennox, 1960). It may also be considered to be somewhat akin to other anticonvulsants like Atrolactamide.



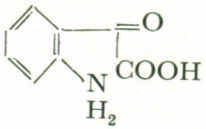
Indigo



Isatin



Atrolactamide



Isatinic acid

Non-toxicity.—The non-toxicity of indolin-2,3-dione, reported in rats by Hidy (1946) and Bruckman and Wertheimer (1947), was considered to be due to its possible catabolism to the non-toxic anthranilic acid (Hilderbrandt, 1903; Kleist, 1903). This conversion has also been seen by Ichihaya *et al.*, (1956) by using rabbit liver enzymes.

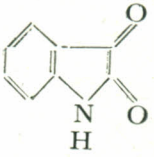
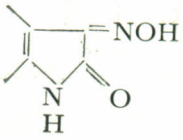
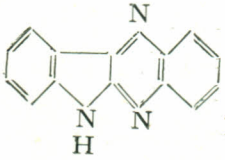
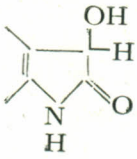
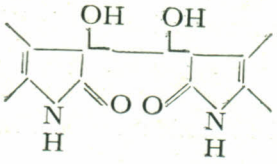
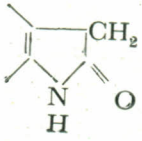
METHODS

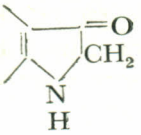
Preparation.—The compounds discussed below were prepared by using standard methods (Elerfield, 1952a; Sumpter and Miller, 1954a).

Screening.—Albino rats of either sex weighing between 75 and 100 g were used in groups of ten animals each per drug for screening the anticonvulsant activity of the compounds by the maximal electroshock seizure (MES) test (Swinyard *et al.*, 1952). Electroshock machine previously described (Gujral *et al.*, 1957b), was employed. Indolin-2,3-dione was tested in doses ranging from 50 to 400 mg/kg whereas its derivatives and analogues were tested at the maximum dose (400 mg/kg). A current intensity of 150MA for a duration of 0.38 sec was found to be quite suitable to produce the typical seizure patterns in all the animals. The electrical stimulus was delivered through two corneal electrodes, moistened with normal saline and secured in position by special clips over the eyelids of the animals, two hours after the administration of the drugs The end point was the abolition of the hind leg tonic extensor component of the seizure. Percentage of the rats protected by each drug was noted.

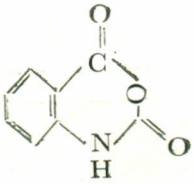
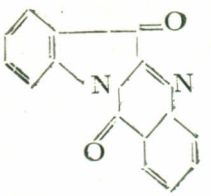
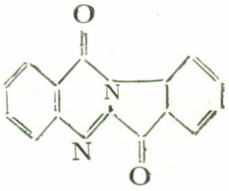
RESULTS AND DISCUSSION

The results of the screening of 17 compounds have been given below.

Sl. No.	Name	Formula	Dose mg/kg	Anticonvulsant activity (percent protection)
1	2	3	4	5
A.	*Parent compound*			
	1. Indolin-2, 3-dione (Isatin)		50	33
			100	50
			200	80
			400	100
B.	*3-Keto-group blocked*			
	2. Isatin-β-oxime		400	0
	3. Indoloquinoxaline		,,	0
C.	*Reduction products*			
	4. Dioxindole		,,	33
	5. Isatide.		,,	0
	6. Oxindole		,,	0

7. Indoxyl		400	33

D. *Oxidation products*

8. Isatoic anhydride (CrO_3-oxidation products).		,,	0
9. Anhydroisatin-α-anthranilide ($KMnO_4$-oxidation product).	 or 		0

E. *N-substituted compounds*

10. N-Methyl-isatin.	R=$-CH_3$	400	0
11. N-Ethyl-isatin.	$-CH_2$	,,	0
12. N-Benzyl-isatin.	$-CH_2.C_6H_5$	,,	0
13. N-Phenyl-isatin.	$-C_6H_5$	,,	0
14. N-Acetyl-isatin.	$-COCH_3$	,,	0
15. N-Benzoyl-isatin.	$-COC_6H_5$	,,	0
16. N-Carbethoxy-isatin.	$-COO-C_2H_5$	,,	0
17. N-Carbethoxymethyl-isatin.	$-CH_2-COOC_2H_5$	,,	0

The results shown above revealed that indolin-2,3-dione possessed a potent anticonvulsant action, 100 per cent protection at 400 mg/kg dose level, against maximal electroshock seizures[1]. Whether this action was really due to the assumptions on which its selection was based can be proved only by further work on its mechanism of action[2].

The inactivity of compounds in which the 3-keto group of indolin-2,3-dione has been blocked by oxime formation or by reaction with phenylene

1. Detailed work on its anticonvulsant spectrum has been reported separately.
2. Its effect on the brain GABA and the 5-HT levels and other studies on its mode of action will be reported separately.

diamine to give indoloquinoxaline, or has been modified by reduction or oxidation seems to show that an intact 3-keto group is essential for the activity. The slight activity of indoxyl, which retains the 3-keto group of the parent molecule, is probably due to the fact that it is an α-keto-amine rather than an α-keto-amide.

Although 2-alkyl-3,1-benzoxazine-4-ones (such as 2-acetyl- and propionyl-anthranils, (unpublished observation Sareen *et al.*) and benzoxazine-2,4-dione (isatoic anhydride) were devoid of any anticonvulsant action, 2-alkyl-3-aryl-quinazol-4-ones, their structural analogues, did possess potent anticonvulsant action (Sareen *et al.*, 1959). Anhydroisatin-α-anthranilide, which can be considered as a 1,2-condensation product of isatin with anthranilic acid on the one hand and as a cyclic analogue of N^3-(2-carboxy-phenyl)-quinazol-4-one on the other, was also inactive.

N-alkylation or acylation of indolin-2, 3-dione seems to inactivate the molecule with respect to its anticonvulsant activity.

In view of the concept of the probable unity of fundamental mechanisms of excitation which produce ectopic ventricular impulses in acute myocardial infarction and those which evoke focal cerebral impulses leading to epileptogenic seizures (Harris and Kokernot, 1950), it is suggested that indolin-2,3-dione may prove to be a potential antiarrythmic agent. Likewise, it may also be of use in hepatic dysfunctions due to ammonia intoxication (Tower, 1960d).

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