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Azabenzoquinolizines with Tranquillizing Activity

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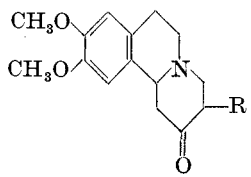
Among the drugs with the ability to produce tranquillization, reserpine and its active analogues are characterized by a unique combination of pharmacological effects. Careful research into the mechanism of reserpine action has led to the discovery that serotonin (5-hydroxytryptamine) is sharply depleted in the brain, intestine and platelets[1, 2a] following reserpine administration. At the same time, urinary excretion of 5-hydroxyindoleacetic acid, the major metabolite of serotonin, is increased considerably. Brodie and co-workers have postulated that the central effects of reserpine are mediated by the release of bound serotonin. These workers have proposed that reserpine interferes with the mechanism by which serotonin is bound in the brain; the serotonin thus released from its binding sites (so-called 'free' serotonin) is rapidly metabolized by monoamine oxidase. This hypothesis, with serotonin as a mediator, is supported by the observation that after significant amounts of reserpine have disappeared from the brain, tranquillization is still observed and the concentration of bound serotonin remains low.

In vitro studies[2b] on platelets also support the release-metabolic degradation theory for serotonin.

It has further been observed that norepinephrine is also sharply depleted in the brain following reserpine administration,[3, 4, 5a] leading some workers to suggest norepinephrine as the prime mediator of reserpine-induced tranquillization. The almost complete depletion of *both* serotonin and norepinephrine in the brain by reserpine at a 1–2 mg/kg dose makes a choice between these two possible mediators very difficult. More recent results[5b] using lower doses of reserpine (0·1 mg/kg) indicated decreases in brain serotonin but increased norepinephrine in the guinea pig. These experiments have been repeated by Brodie[5c]

and found to be in error. Much work clearly remains to be done in this area.

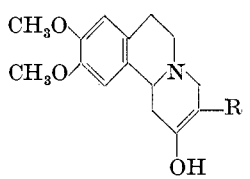
These interesting biochemical effects on brain amines had been observed only with the naturally occurring Rauwolfia alkaloids and certain of their derivatives—only those which cause tranquillization. In 1957, a class of totally synthetic organic compounds, the tricyclic compounds known as benzoquinolizines, were announced to have these properties of release-type tranquillizers. In a series of publications, Pletscher and co-workers[6a, b, c] have described the benzoquinolizines of structure I. These 2-oxo-



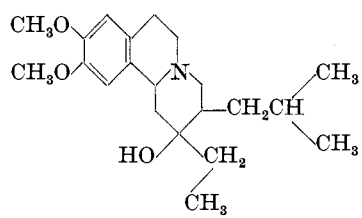
(I)

benzo-(a)-quinolizines are reported to have a reserpine-like central action but are less potent, and have little or no effect on the blood pressure.[6e] These compounds, like reserpine, cause release of the brain amines, although to a lesser degree. The most potent reported derivative of I (where R = isobutyl)* is much less potent than reserpine and far shorter acting.[6c, d] It liberates only part of the brain serotonin, even at high doses, but produces all the gross signs of tranquillization in various animal species. Several clinical papers have recently appeared demonstrating that this drug is also an active psychosedative in man.[7]

It was of interest to explore further this area of release-type tranquillizers with the aim of determining what structural features



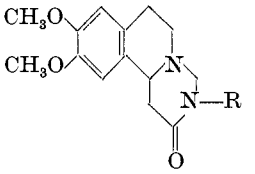
(IA)



(IB)

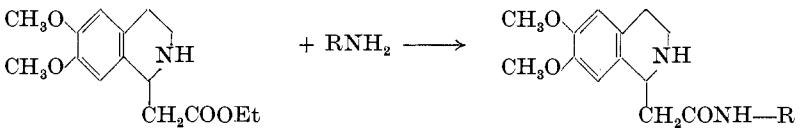
* Nitoman ® (tetrabenazine).

are necessary in these benzoquinolizines for significant release of brain amines. As a starting point, attention was directed to the piperidone ring of this heterocycle. The polar nature of the ketone function in I, together with the possible formation of the favoured enol IA, suggested that this grouping might play an important role in the mechanism by which these compounds act in the brain.* Accordingly, the ketonic function in I was replaced by an amide function in order to study the effect of this variation on the biological activity of these compounds. This paper describes the preparation and biological evaluation of the resulting tricyclic lactams—the 3-alkylaza-2-oxo-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo-(a)-quinolizines (II).



(II)

To prepare this previously unknown heterocyclic system, a two-step procedure was employed. Condensation of the known 1-carbethoxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (III) with primary amines gave the corresponding *N*-alkyl-α-(6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolyl)-acetamides (IV) in good yields. In this step, with a few exceptions, the carbethoxymethyltetrahydroisoquinoline III was heated in the presence of an

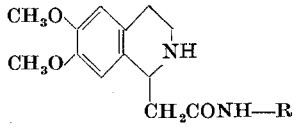


(III) (IV)

excess of the appropriate primary amine as solvent in order to discourage intermolecular condensation of the amino ester III with itself. Table I summarizes the reaction conditions and properties

* The alcohol IB derived from I has been reported [Pletscher, A., Besendorf, H. and Gey, K. *Science*, **129**, 844 (1959)] to be approximately ten times *more* potent than I. Perhaps the hydroxyl functions in IA and IB are important in the mechanism by which these compounds produce tranquillization.

Table I. *N*-Alkyl-α-(1-tetrahydroisoquinolyl) acetamides



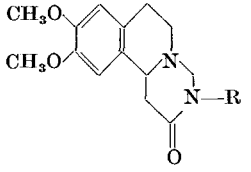
(IV)

	R	m.p., °C	Yield, %	Recryst. solvent	Reaction temp., °C	Catalyst
1.	*n*-butyl	87–88	66[b]	benzene–cyclohexane	78	none
2.	isobutyl	107–108	65	benzene–cyclohexane	68	[a]
3.	β-hydroxyethyl	114–115	75	benzene	80	none
4.	allyl	92–94	67	ether	53	[a]
5.	—H	171·5–172	60[b]	ethanol	25 (methanol)	none
6.	β-dimethylaminoethyl	88–92	29	benzene–hexane	130	[a]
7.	phenethyl	114–116	92	—	150	[a]
8.	γ-hydroxypropyl	115–119	89	—	125	none
9.	γ-methoxypropyl	liquid	90	—	116	[a]
10.	γ-dimethylaminopropyl	liquid	80	—	159	[a]
11.	benzamido	173·5–175·5	30[b]	benzene	78 (ethanol)	[a]
12.	isoamyl	74–76	70	ether	95	[a]
13.	benzyl	129–132	90	ether	150	[a]

[a] See experimental section.

[b] 100 mg of *p*-toluenesulphonic acid per 0·025 mole of III.

Table II. 3-Substituted-aza-2-oxo-benzo-(a)-quinolizines

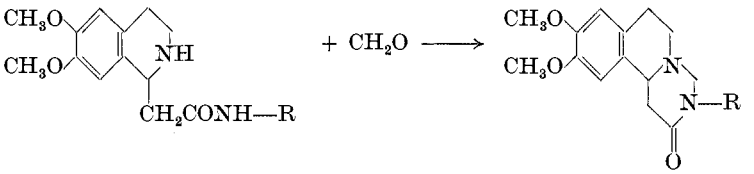


(II)

	R	m.p., °C	Yield, %	Recryst. solvent	Analysis, %					
					Calcd.			Found		
					C	H	N	C	H	N
1.	*n*-butyl	114–116	72	ethyl acetate	67·89	8·23	8·80	67·83	8·20	8·93
2.	isobutyl	104–106	59	benzene–hexane	67·89	8·23	8·80	68·01	8·30	8·61
3.	*p*-chlorobenzyl	142–145	33	ethyl acetate	65·19	5·99	7·24	65·17	6·06	7·39
4.	β-hydroxyethyl	146–147	69	isopropyl alcohol	62·72	7·24	9·15	63·03	7·29	9·34
5.	allyl	86–89	28	benzene–cyclohexane	66·10	7·95	9·64	66·25	8·06	9·86
6.	β-(3,4,5-trimethoxy-benzoyloxy)ethyl	116–119	69	benzene–hexane	62·38	6·45	5·60	62·06	6·52	5·28
7.	phenethyl	172–172·5	82	—	72·10	7·15	7·65	71·88	7·21	7·87
8.	γ-hydroxypropyl·HCl	165–166·5	90	ethanol–ether	57·21	7·06	7·85	56·88	7·28	7·75
9.	γ-methoxypropyl	103–105	58	isopropyl alcohol	64·64	7·84	8·38	64·58	7·98	8·55
10.	benzamido	206–207	47	isopropyl alcohol	66·13	6·08	11·02	65·97	6·11	11·16
11.	isoamyl	90·5–91·5	85	methylcyclohexane	68·64	8·49	8·43	68·70	8·58	8·78
12.	benzyl	115–118	87	ether	71·57	6·86	7·95	71·23	7·05	8·34
13.	γ-dimethylaminopropyl	100–103	55	cyclohexane–benzene	65·68	8·41	12·09	65·88	8·43	12·17
14.	β-dimethylaminoethyl	80–82	74	hexane–benzene	64·84	8·16	12·60	65·09	8·52	12·35
15.	—H	178–181	65	ether	64·11	6·91	10·68	63·69	7·06	10·13

of the *N*-alkyl-α-(1-tetrahydroisoquinolyl)-acetamides (IV) prepared. Detailed conditions for a typical preparation and some exceptional procedures are given in the experimental section.

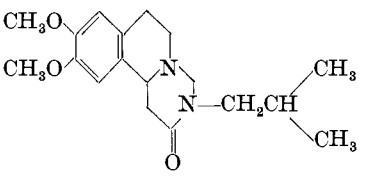
Each compound of type IV was then cyclized to the desired 3-aza-2-oxo-benzo-(a)-quinolizine (II) in good yield with formaldehyde in basic solution. The pH and temperature of these reactions were carefully controlled in order to obtain optimum yields. A summary of the physical properties and yields of azaquinolizines (II) appear in Table II, and a typical cyclization procedure is given in the experimental section.



(IV) (II)

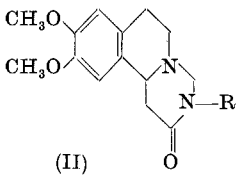
Biological evaluation of these azaquinolizines (II) consisted of measuring by a spectrofluorometric method[8] the percentage fall in serotonin and norepinephrine levels in the rabbit brain two hours after intravenous administration of solutions of II. Two hours was arbitrarily chosen as the time of measurement of depletion of both brain amines, based on reported results with I.[6a, b]

Table III presents the effects on the brain amines after administering 50 mg/kg of II to rabbits. The results in most cases represent the average of six determinations and are expressed as the percentage decrease from normal values. In only two cases (II-7, II-11) did toxic effects require a reduction in dose (25 and 35 mg/kg, respectively). From the visible tranquillization produced in the rabbit, compound II-2 appeared to be the most active of the series; at the same time, this compound was one of



(II-2)

Table III. Effect of azabenzoquinolizines on brain amines



(II)

R	Rabbit brain stem, 50 mg/kg 2-h duration % Reduction from normal values	
	serotonin	norepinephrine
1. *n*-butyl	0	21
2. isobutyl	23	18
3. *p*-chlorbenzyl	0	0
4. β-hydroxyethyl	6	+5
5. allyl	19	18
6. β-(3,4,5-trimethoxybenzoyloxy)-ethyl	+10	0
7. phenethyl (25 mg/kg)	18	8
8. γ-hydroxypropyl	8	0
9. γ-methoxypropyl	15	14
10. benzamido	20	14
11. isoamyl (35 mg/kg)	7	18
12. benzyl	4	0
13. γ-dimethylaminopropyl	9	10
14. β-dimethylaminoethyl	4	14
15. hydrogen	+23	+18

the stronger depressors of brain amines. Although the nature of the carbonyl function is unquestionably changed by the addition of a nitrogen atom, it is interesting to note that in this series, as in structure I, the isobutyl side chain (II-2) produced maximum activity.

Further studies were undertaken in order to determine the time required for maximum reduction in both serotonin and norepinephrine after administering II-2. Fig. I illustrates graphically that the brain serotonin concentration is reduced by 33 per cent at 15 min with a gradual return to normal over a 4-hour period.

Fig. 2 presents the data for norepinephrine concentrations after

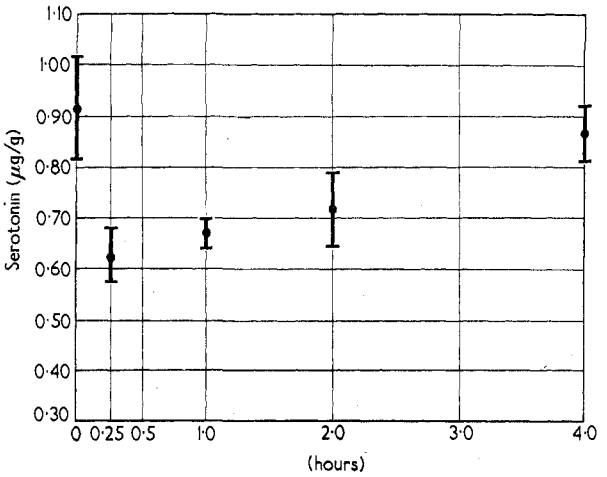


Fig. 1. Serotonin concentration in rabbit brain after 50 mg/kg of II-2. Determinations made on six animals at each time; bars indicate standard errors.

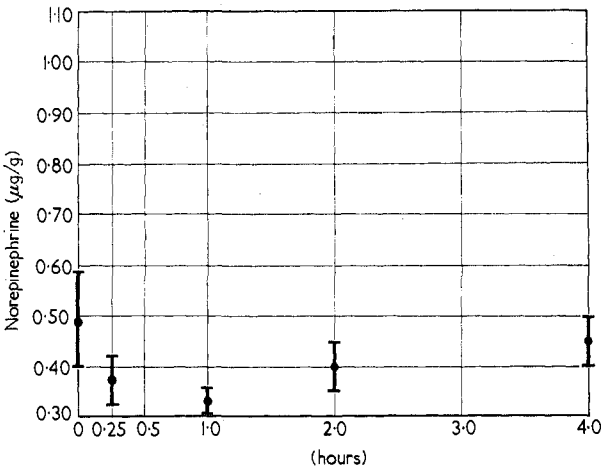


Fig. 2. Norepinephrine concentration in rabbit brain after 50 mg/kg of II-2. Determinations made on six animals at each time; bars indicate standard errors.

administration of II-2; here, a maximum reduction of 22 per cent was observed at 1 hour. The normal values for these brain amines are given in the figures at zero time. Outwardly, the rabbits given 50 mg/kg of II-2 exhibited ptosis and were quiescent. These effects were absent, however, in other derivatives of II. Unlike the long-acting reserpine, the effects of which persist long after detectable quantities of drug have disappeared from the brain, the effects of II-2 are of short duration.

Brain Concentration of II-14

In order to ascertain whether any derivatives of II might interfere with the spectrofluorometric analysis for serotonin or norepinephrine, each compound was carried through the extraction procedures used in the brain amine assays and the resulting solutions were then read on the spectrofluorometer. Of those listed in Table III, only compounds 13 and 14 were found to exhibit spectra which interfered with the spectrum of serotonin. By standard techniques, the contributions to the spectrum due to these compounds could be calculated and the serotonin assayed in their presence. Since compound 14 survived the extraction procedures[8] used in assaying for the brain amines, it provided an opportunity for showing semi-quantitatively that these compounds were crossing the blood–brain barrier. By comparison with solutions of known concentration, it was estimated that 2–4 μg/g of compound 14 was present in the brain tissue at the time of sacrifice (2 h).

Effect of Pretreatment with an MAO Inhibitor

To compare further the mechanism of action of the azabenzoquinolizines with that of reserpine, animals were pretreated with nialamide,* a monoamine oxidase inhibitor, for four days at 10 mg/kg. By analogy with reserpine, pretreatment with a monoamine oxidase inhibitor was expected to antagonize the sedative effect of the drug by inactivation of the enzyme that metabolizes the amines released from binding sites in the brain. This effect

* Niamid ®

was realized. Administration of 50 mg/kg of II-2 to nialamide-pretreated rabbits produced no sedation. Instead, these animals were alert and responsive, with the only observable effect being dilation of the pupils.

These azabenzoquinolizines, then, represent a second class of synthetic organic compounds which are capable of reducing the brain concentrations of serotonin and norepinephrine while producing the gross symptoms of reserpine-like sedation. Although most were only weakly active, one particular member of this series was shown to be markedly superior to other members as a tranquillizer in the rabbit.

Experimental

All melting points are corrected.

The preparations of the 3-azabenzo-(a)-quinolizines were carried out by a general two-step procedure which is illustrated below for the 3-(*n*-butyl)-aza derivative.

N-(n-*Butyl*)-(*6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolyl*)-*acetamide* (*IV-1*). In a 35-ml round-bottomed flask, equipped with water condenser and drying tube, was placed 1-carbethoxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline[9] (1·4 g, 0·005 mole) and *n*-butylamine (10 g, 0·137 mole). The reaction mixture was placed under a nitrogen atmosphere and refluxed for 24 h. Then, distillation of excess butylamine gave a residual liquid which slowly crystallized. Recrystallization from benzene–cyclohexane yielded 1·01 g (66 per cent) of a white solid, m.p. 87–88°.

Anal. Calcd. for $C_{17}H_{26}N_2O_3$: C, 66·64; H, 8·55; N, 9·14. Found: C, 66·66; H, 8·55; N, 9·04.

Certain high-boiling amines were not reacted at reflux and some reactions required an acid catalyst for best yields (see Table I for appropriate conditions).

3-(n-*Butylaza*)-*9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-2-oxo-benzo-*(*a*)-*quinolizine* (*II-1*). In an Erlenmeyer flask immersed in a water bath at 60° were placed *N*-(*n*-butyl)-α-(6,7-dimethoxy-1,2,3,4-tetrahydro-1-isoquinolyl)-acetamide (6·0 g, 0·02 mole), ethanol (60 ml) and 5 per cent sodium hydroxide solution (5 ml). To the resulting yellow solution was added 37 per cent aqueous formaldehyde (4·8 g, 0·06 mole) and the solution stirred at 60°

for 2 h. Evaporation under reduced pressure yielded an oil, which was dissolved in benzene (100 ml). The benzene solution was washed twice with 100-ml portions of 2 N HCl, the acid washes were combined, immediately made basic with 10 per cent sodium hydroxide, and the basic aqueous layer was extracted with benzene. After drying and evaporation, the benzene extracts yielded 4·5 g (72 per cent) of a white solid which on recrystallization from ethyl acetate melted at 114–116°.

Anal. Calcd. for $C_{18}H_{26}N_2O_3$: C, 67·89; H, 8·23; N, 8·80. Found: C, 67·83; H, 8·20; N, 8·99.

Some later experiments on other derivatives of II indicated that yields of cyclized product were optimum when the basicity of the reaction mixture was adjusted to pH 11 by addition of 5 per cent sodium hydroxide.

α-(6,7-Dimethoxy-1,2,3,4-tetrahydro-1-isoquinolyl)-acetamide (IV-5). After cooling to 0°, 200 ml of absolute methanol was saturated with ammonia. To this solution was added 1-carbethoxymethyl-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (8·0 g, 0·028 mole) and stirring at 0° was continued for 4 h. The solution was then allowed to stand at room temperature for 48 h. Evaporation of solvent under reduced pressure yielded 4·2 g (60 per cent) of a solid, which after recrystallization from ethanol melted at 171·5–172°.

Anal. Calcd. for $C_{13}H_{18}N_2O_3$: C, 62·38; H, 7·25; N, 11·19. Found: C, 62·24; H, 7·14; N, 10·90.

N-Benzoyl-N-[α-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl)acetyl]-hydrazine (IV-11). In a round-bottomed flask fitted with a water condenser and magnetic stirrer was placed 1-carbethoxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (6·40 g, 0·023 mole), benzhydrazide (8·15 g, 0·060 mole), *p*-toluenesulphonic acid (100 mg) and absolute ethanol (35 ml). The resulting solution was placed under a nitrogen atmosphere and refluxed for 48 h. Evaporation of the solvent under reduced pressure yielded an oil which slowly crystallized. Recrystallization from benzene–ether yielded first some benzhydrazide, and then further crops of crystals were obtained; yield, 2·6 g (30 per cent), m.p. 174–175·5°. Repeated attempts to prepare a sample of analytical purity failed. Paper chromatography showed a trace of impurity which moved differently from either of the starting materials. No

further attempt was made to identify this trace impurity. The crude sample was therefore cyclized with formaldehyde to II-10 which was analyzed (Table II).

3-Aza-3-(β-3,4,5-trimethoxybenzoyloxyethyl)-1,2,3,4,6,7-hexahydro-11bH-2-oxo-9,10-dimethoxy-benzo-(a)-quinolizine (II-6). A solution of trimethoxybenzoyl chloride (3·1 g, 0·013 mole) (m.p. 78–80°) in dry pyridine (30 ml) was prepared in a 150-ml round-bottomed flask protected by a drying tube. After stirring for 5 min, 3-aza-3-(β-hydroxyethyl)-1,2,3,4,6,7-hexahydro-11bH-2-oxo-9,6-dimethoxybenzo-(a)-quinolizine (3·38 g, 0·013 mole) was added in one portion. The resulting dark solution was stirred at 60° for 2 h, then poured into an ice–water mixture. Extraction of the water with chloroform, then successive washing of the combined chloroform extracts with water, 5 per cent sodium bicarbonate, and again water, yielded a light yellow organic layer. After drying over sodium sulphate and removal of solvent, recrystallization from benzene–hexane yielded 3·85 g (69 per cent) of white needles, m.p. 119–120°.

Anal. Calcd. for $C_{26}H_{32}N_2O_8$: C, 62·38; H, 6·45; N, 5·60. Found: C, 62·08; H, 6·52; N, 5·28.

Summary. A series of new heterocyclic compounds have been prepared and tested as tranquillizers in the rabbit. Some of these new 3-alkylaza-2-oxo-9,10-dimethoxy·1,2,3,4,6,7·hexahydro·11bH·benzo-(a)-quinolizines cause tranquillization and at the same time reduce the concentration of brain serotonin and norepinephrine. One particular derivative, the 3-isobutylaza-2-oxo-9,10·dimethoxy-1,2,3,4,6,7-hexahydro-11bH·benzo-(a)·quinolizine (II-2), proved to be the best of the series in its ability to produce mild reserpine-like activity. A time study showed II-2 to be a short-acting compound, with greatest reduction in the concentration of brain serotonin at 15 minutes. From all outward appearances, animals treated with II-2 appeared tranquillized for a short time. Like reserpine, the tranquillizing effects of II-2 were antagonized by pretreatment with the monoamine oxidase inhibitor, nialamide. Though comparatively quite weak, these compounds represent a second class of synthetic medicinals which, like reserpine, produce tranquillization accompanied by a decrease in brain amine concentrations.

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