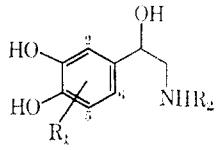
TABLE II

THE CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY RING METHYL AND METHOXY DERIVATIVES OF NOREPINEPHRINE[a]



No.	R_1	R_2	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
14[b]	...	H	2.5	33
15[b]	...	CH_3	2.5	36
16	...	$CH(CH_3)_2$	10	86
17[c]	2-CH_3	H	10	55
18[c]	2-CH_3	CH_3	10	88
19[c]	2-CH_3	$CH(CH_3)_2$	10	76
20[c]	2-OCH_3	H	10	87
21[c]	2-OCH_3	CH_3	10	86
22[c]	2-OCH_3	$CH(CH_3)_2$	10	81
23[c]	5-CH_3	H	10	32
24[c]	5-CH_3	CH_3	10	74
25[c]	5-CH_3	$CH(CH_3)_2$	10	89
26	5-OCH_3	H	10	94
27[c]	6-CH_3	H	10	30
28[c]	6-CH_3	CH_3	10	82
29[c]	6-CH_3	$CH(CH_3)_2$	10	100
30	6-OCH_3	H	5	80
31[c]	6-OCH_3	$CH(CH_3)_2$	10	100

[a] Assay as described[2] with five mice per group. Standard error ±5%. Hydrochloride salts unless otherwise indicated. [b] Bitartrate salt. [c] Provided by C. H. Boehringer Sohn.

tion of this amino acid (50 mg/kg sc), the level of norepinephrine in brain[8] had decreased to 60 ± 15% of normal. This same dose depleted cardiac norepinephrine-^{3}H to a level corresponding to 34 ± 5% of control. 3,4,5-Trihydroxyphenylalanine (**13**) has now been reported to deplete endogenous norepinephrine in both heart and spleen[9] and brain.[10]

Other ring methyl and methoxy analogs of norepinephrine have been tested for releasing activity (Table II, which includes (nor)epinephrine and isoproterenol (**14–16**) for comparison). None of the analogs show the very high activity of 3,5-dihydroxy-4-methoxyphenyl derivatives. All of these methoxy derivatives have low activity in agreement with earlier relations between ring methoxylation and lack of norepinephrine-releasing activity.[2,4] The 3,5-dihydroxy-4-methoxy derivatives provide the sole exception to this generalization. Norepinephrine derivatives with methyl substituents in the ring *retained* appreciable activity (**17**, **23**, **27**) which was found to be reduced by N-alkylation.

Studies are now in progress on the pharmacological effects of 3,5-dihydroxy-4-methoxyphenylalanine and 3,4,5-trihydroxyphenylalanine in both the cardiovascular and the central nervous systems.

Acknowledgments.—The authors would like to express their gratitude to the various pharmaceutical firms which generously provided various amines and amino acids. The skilled assistance of Mrs. Louise Atwell is gratefully acknowledged.

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The Chemorelease of Norepinephrine from Mouse Hearts. IV. Structure–Activity Relationships. Reserpines and Yohimbines

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Received November 16, 1967

In the last 10 years the effect of structural modifications on the sedative and antihypertensive properties of reserpine have been intensively studied (for an excellent review see ref 2). Since reserpine apparently elicits its effects through depletion of biogenic amines, in particular norepinephrine, one method of probing structure–activity correlations in this class of compounds is to determine whether they cause depletion of norepinephrine. The use of an assay based on prelabeled cardiac stores of norepinephrine-^{3}H permitted the scrutiny of a large variety of compounds for their ability to deplete this important neurotransmitter. In a preliminary investigation on various drugs,[3b] it was noted that another *Rauwolfia* alkaloid, yohimbine, also caused depletion of cardiac norepinephrine-^{3}H. The norepinephrine-^{3}H-releasing activity of reserpine, yohimbine, and a variety of congeners have now been studied (Tables I and II). The method employed possesses the great advantages of requiring only 1–2 mg of sample and of providing definitive results within a short time.

Variations in the ester or other oxygen substituents at position 18 of reserpine (**1–7**) had little effect on the degree of norepinephrine-^{3}H release in correspondence with pharmacological data.[4] Methyl reserpate (**8**) which contains a free 18-hydroxyl group retained some activity in this assay in contrast to its reported lack of reserpine-like action.[4] 3-Epi-α-yohimbine (**37**), in which the 18-oxygen group is replaced by hydrogen, did not cause release of norepinephrine-^{3}H, nor did it possess marked sedative activity (Table II). Of the congeners tested which are epimers of reserpine at position 18 (**18–20**), all retained some, if not all, of their norepinephrine-^{3}H-releasing activity. It has been reported that methyl 18-epireserpate (**18**) has sedative activity,[2] while 18-epireserpine is inactive.[5]

Certain variations in the aromatic substituents caused diminution of norepinephrine-^{3}H-releasing activity (**10**, **11**, and **23**), while other alterations such as the removal of the 11-methoxy substituent of reserpine

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TABLE I

CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY RESERPINE AND RELATED COMPOUNDS[a]

No.	Compound	Sedation[b]	Norepinephrine-^{3}H in heart, % of control
	Variations in 18-Substituent		
1	Reserpine (methyl reserpate 18-O-TMB)[c]	+	12
2	Rescinnamine (methyl reserpate 18-O-TMC)[c]	+	27
3	Methyl reserpate 18-O-(3,5-dimethoxy-4-hydroxybenzoate)	+	25
4	Syrosingopine [methyl reserpate 18-O-(3,5-dimethoxy-4-O-carboethoxybenzoate)]	+	28
5	Methyl reserpate 18-O-acetate	+	24
6	Methyl reserpate 18-O-methyl ether	+	25
7	Methyl reserpate 18-O-ethyl ether maleate	+	24
8	Methyl reserpate	+	45
	Variations in Aromatic Substituents		
9	Deserpidine (11-desmethoxyreserpine)	+	20
10	10-Acetylreserpine	−	59
11	12-Acetylreserpine	−	70
	Variations in 17-Substituent		
12	Raunescine (17-desmethyldeserpidine)	+	23
13	Isoraunescine (17-O-TMB, 18-OH)	−	53
	Stereoisomers		
14	Methyl isoreserpate	−	104
15	Methyl isoreserpate 18-O-methoxyethyl ether	−	108
16	Methyl 16-epireserpate 18-O-TMB[c]	−	82
17	Methyl 16,17-diepireserpate 18-O-TMB[c]	−	85
18	Methyl 18-epireserpate	+	41
19	Methyl 18-epireserpate 18-O-methyl ether HCl	+	27
20	Methyl $\Delta^{16,17}$-17-desmethoxy-18-epireserpate 18-O-acetate	−	67
21	Methyl 16,17,18-triepireserpate 18-O-TMB[c]	−	85
22	Methyl 16,17,18-triepireserpate 18-O-acetate	−	85
	Other Structural Modifications		
23	Methyl N_a-methylreserpate	−	91
24	Renoxidine (reserpine N_b-oxide)	+	55
25	Reserpic acid lactone	−	90
26	Raujemidine ($\Delta^{19,20}$-reserpine)	−	52
27	Raunitidine	−	105
28	Reserpiline oxalate	−	96
29	Ajmaline	−	83
30	Tetraphyllicine	−	83
31	Sarpagine	−	98
32	Perakine	−	82
33	Corynantheine	−	103

[a] Assay as described in ref 3a, dose 5 mg/kg, five mice per group, standard error ±5%. [b] Symbols: + indicates pronounced sedation, ptosis; − indicates no effect or mild sedation. [c] TMB, 3,4,5-trimethoxybenzoate; TMC, 3,4,5-trimethoxycinnamate.

to form deserpidine (**9**) were without effect. The inactivation of reserpine by N_a-alkylation, as here demonstrated for **23**, has been reported.[6]

Renoxidine (**24**), the N-oxide of reserpine, elicited norepinephrine-^{3}H release and has been reported to have reserpine-like activity.[7] Quaternization of this basic nitrogen, however, inactivates reserpine.[4]

TABLE II

CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY YOHIMBINE AND RELATED COMPOUNDS[a]

No.	Compound	Sedation[b]	Norepinephrine-^{3}H in heart, % of control
34	Yohimbine[c]	−	60
35	Corynanthine[c]	−	69
36	β-Yohimbine	−	93
37	Pseudoyohimbine	−	103
38	3-Epi-α-yohimbine	−	92
39	α-Yohimbine	−	94
40	17-Yohimbone	−	109
41	Yohimbane	−	100
42	16-α-Hydroxyyohimbane	−	88
43	17-β-Hydroxyyohimbane	−	73
44	17-α-Chloroyohimbane	−	89
45	N_a,O-Diacetylyohimbine	−	85

[a] Assay as described in ref 3a; dose 5 mg/kg. Five mice per group, standard error ±5%. [b] Symbols: + indicates profound sedation, ptosis; − indicates no effect or mild sedation. [c] Both yohimbine and corynanthine caused sedation, ptosis, and increased release of norepinephrine-^{3}H at higher dosages.

Both 17-methoxy (deserpidine, **9**) and 17-hydroxy (raunoscine, **12**) derivatives caused release of norepinephrine-^{3}H. The 17-hydrogen analog, which was not tested, has been reported to retain reserpine-like activity.[8] Even isoraunescine (**13**), which has a **17**-O-trimethoxybenzoate group, caused some release of norepinephrine-^{3}H.

It is well known that variations in ester function at position 16 have little effect on pharmacological activity,[6] while the free acid[9] and reserpamide[6] are devoid of sedative or hypotensive properties. Reserpic acid lactone (**25**), 16-epireserpine (**16**), and 16,17-diepireserpine (**17**) did not cause significant release of cardiac norepinephrine-^{3}H. The lactone contains the C–D–E rings in the *trans–trans–cis* conformation rather than the preferred *cis–trans–cis* conformation of reserpine and lacks reserpine-like activity.[4]

Inversion of all three substituents in ring E led to derivatives (**21**, **22**) which were relatively inactive in releasing norepinephrine. These compounds have some sedative activity.[5]

The importance of ring E is also shown by the lack of releasing activity in *Rauwolfia* alkaloids containing a heterocyclic E ring (**27**, **28**). Raujemidine (**26**), which contains a 19,20 double bond, is still moderately active in causing release of norepinephrine-^{3}H. Molecular models of raujemidine reveal a three-dimensional arrangement similar to that of reserpine.[2]

Other *Rauwolfia* alkaloids, such as the indoline bases, ajmaline, tetraphyllicine, and sarpagine (**29–31**) were nearly inactive as releasing agents. Corynantheine (**33**) and perakine (**32**) which do not have ring E of reserpine were also inactive. Isomerization of the 3 position, in agreement with the results of other investigators,[2] leads to the totally inactive isoreserpine series (**14**, **15**).

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In view of these findings it is surprising that yohimbine and corynanthine (**34**, **35**), which have the opposite configuration of reserpine at position 3 and have a *trans*-D–E ring junction, caused significant release of cardiac norepinephrine-^{3}H. The depletion of norepinephrine stores in brain by yohimbine has been reported.[10]

As in the reserpine series, the ability to release cardiac norepinephrine was lost on epimerization of position 3 (pseudoyohimbine, **37**). Quaternization of the basic nitrogen resulted in loss of activity[3b] as is the case with reserpine. Releasing activity was also observed with 17-β-hydroxyyohimbane (**43**). Study of molecular models did not reveal any obvious spatial correlations between reserpine and yohimbine. In addition, pretreatment with bretylium or cocaine effected drug-induced release (*cf.* Figure 7 in ref 11) differently for reserpine and yohimbine (Table III).

TABLE III
EFFECT OF COCAINE AND BRETYLIUM IN NOREPINEPHRINE RELEASE INDUCED BY RESERPINE AND YOHIMBINE[a]

Blocking agent	Releasing agent	Norepinephrine-^{3}H in heart, % of control
...	...	100
Bretylium	...	94
Cocaine	...	95
...	Reserpine	21
Bretylium	Reserpine	53
Cocaine	Reserpine	23
...	Yohimbine	60
Bretylium	Yohimbine	65
Cocaine	Yohimbine	35

[a] Cocaine or bretylium (20 mg/kg) administered subcutaneously 30 min after prelabeling mouse heart by the intravenous administration of 5 μCi of norepinephrine-^{3}H. Reserpine (1 mg/kg) or yohimbine (5 mg/kg) were administered subcutaneously at 60 min. Mice were sacrificed at 180 min and norepinephrine-^{3}H in heart was determined as described previously.[3a]

Bretylium partially blocked reserpine-induced release but did not significantly alter the release caused by yohimbine. Cocaine had no effect on reserpine-induced release, but markedly potentiated the release induced by yohimbine. The yohimbine–cocaine combination produced severe sympathomimetic effects in mice including piloerection, salivation, and hyperactivity. A similar increase in the toxicity of yohimbine is elicited by other drugs, such as imipramine, which interfere with neuronal uptake of norepinephrine.[10] These observations suggest a different mode of action for reserpine and yohimbine. Continuing studies in this laboratory indicate that the abilities of reserpine and yohimbine to release cardiac norepinephrine are not additive, but rather are partially competitive.[12]

Acknowledgments.—The authors would like to express their deep gratitude to Dr. W. I. Taylor of Ciba for his generosity in providing the majority of these compounds and to Dr. M. von Strandtmann of Warner-Lambert Research Institute for certain yohimbane derivatives. The technical assistance of Mrs. Louise Atwell is gratefully acknowledged.

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A Study of the Toxicity of Several Cyclopentylamino Acid Analogs

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Received December 18, 1967

The antibacterial action of 1-aminocyclopentanecarboxylic acid (ACPC)[1–3] and the toxic action in mammals have been reported.[4] This note describes three homologs of ACPC, namely 1-amino-2,5-dimethylcyclopentanecarboxylic acid (I), 1-amino-2-methylcyclopentanecarboxylic acid (II), and 1-amino-3-methylcyclopentanecarboxylic acid (III), and their toxic action on bacteria and mammals.

It was found that III is about 1000 times more toxic to *Escherichia coli* 9723 than ACPC. Compound II is much less toxic than ACPC and I showed no toxicity at all. Of 18 natural amino acids investigated, it was found that isoleucine, leucine, and valine were more powerful reversal agents for III than the others. In each case, elevated concentrations of the natural amino acids caused greater toxicity reversal (see Table I). Smaller differences in concentration gave inconsistent results as the difference in reversal potential was too small.

Concerning toxicity to mice, it was observed that with ACPC (see Figure 1), a remarkable decrease in weight occurred with subsequent death of the animals in 9 days. With III, the animals showed only a minor decrease in weight, with an abrupt weight increase beginning the 4th day after the end of the injections. The other compounds examined were similar to the saline controls.

Experimental Section

Syntheses.—The amino acids were synthesized from the corresponding ketones *via* the hydantoins by a procedure previously described.[5] Compound II was prepared in this manner (mp 295–300°) by L. Nicole.[6] The data of the synthesis of the other compounds appear in Table II.

Bacterial Studies.—A stock culture of *Escherichia coli* 9723 was employed. The salts-glucose medium described by Anderson[7] was modified as described previously[2] before use. The inoculation and incubation methods employed were as described previously.[2] The compounds to be tested were weighed into sterile test tubes and dissolved in sterile water. The solutions were adjusted to pH 7 and added aseptically to the assay tubes. A dilute microbial cell suspension with log-phase cells was utilized for inoculation of the assay; one drop was added to each tube. The incubation was at 37° for 18 hr. The amount of growth was determined turbidimetrically by means of a nephelometer. Utilizing the concentration scale shown in Table III, the toxicity levels for the compounds are indicated. Compound I was not toxic at the highest concentration that solubility would allow in this technique.

Attempts were made to reverse the toxicity of III by 18 natural amino acids: L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-

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