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Letters to the Editor

Spectroscopic studies of some promazine metabolites and 3-hydroxypromazine

SIR,—Recently (Beckett & Curry, 1963), it was shown that the absorption spectra of the semiquinones formed by dissolving phenothiazine compounds in 50% sulphuric acid, could be used to identify the parent compounds. Particular consideration was given to hydroxylated promazine derivatives, because of their importance as possible biotransformation products of promazine. At that time, only three of the four possible monohydroxylated derivatives were available as reference compounds, and it was necessary to predict the spectroscopic properties of 3-hydroxypromazine. We now report the verification of these predictions.

The 3-hydroxypromazine sample (m.p. 143–143°) ran as a single compound in five solvent systems (chloroform:ethanol, 90:10; chloroform:diethylamine, 98:2; and chloroform alone, all on alumina plates, and n-butanol:methanol:formic acid:water, 40:40:2:18; and ethanol:acetic acid:water, 50:30:20; both on silica gel plates). The infra-red spectrum of the compound was consistent with its stated structure. The ultra-violet absorption spectrum of the unoxidised material was similar to those of 1-, 2-, and 4-hydroxypromazines previously reported. In solution in 50% sulphuric acid the absorption spectrum had peaks at 278, 343, 372 and 568 mμ, differing from the corresponding spectrum of 2-hydroxypromazine in the presence of the peak at 372 mμ. (See Fig. 1).

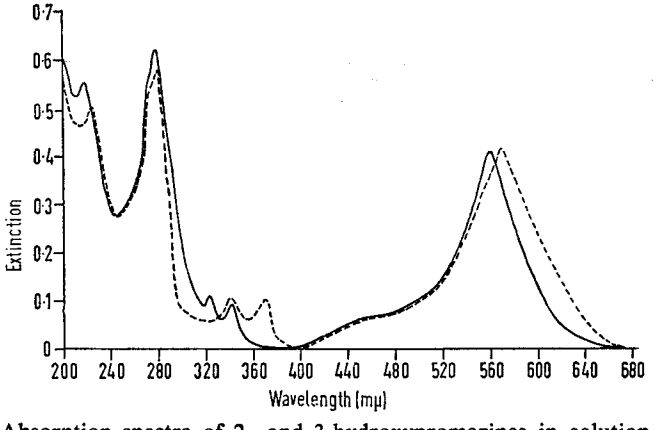


FIG. 1. Absorption spectra of 2- and 3-hydroxypromazines in solution in 50% sulphuric acid, recorded on a Unicam SP800 spectrophotometer. Dilution of the solution for the ultra-violet spectral record to one-tenth of the concentration for the visible spectrum, was effected at approx. 320 mμ. The minor peak at 325 mμ in the spectrum of 2-hydroxypromazine is additional to those previously reported (Beckett & Curry, 1963).

Key, —— 2-hydroxypromazine, - - - - 3-hydroxypromazine.

From urine of psychiatric patients receiving promazine, four glucuronides (C and D, as major and A and B as minor components) were isolated from the main glucuronide fraction, by continuous electrophoresis, paper chromatography, column chromatography and preparative thin layer chromatography.

They were found to be hydroxy primary amine sulphides by spot tests, e.g., ninhydrin +ve, sodium nitroprusside −ve, ferric chloride +ve and sodium periodate +ve. The Rf values of A, B, C and D, with the solvent system n-butanol : methanol : formic acid 98% : water, 40 : 40 : 2 : 18; on thin layer silica gel plates were respectively 77, 75, 67 and 57 (3-hydroxypromazine, Rf = 60).

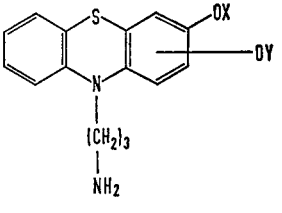
TABLE 1. ABSORPTION MAXIMA OF CERTAIN PROMAZINE DERIVATIVES IN 50% H_2SO_4 SOLUTION

Compound	λmax (mμ)					
2-hydroxypromazine	278	325	343		(440)	558
3-hydroxypromazine	278		343	372	(450)	568
3-methoxypromazine	279		342	372	(450)	565
3,7-dimethoxyphenothiazine ..	297		351	383		592
A	277		?	?	?	547–557
B	277		343	(368)	(450–470)	554
C	277		343	370	(450–480)	552
D	277		343	370	(450–480)	552
aglycone of B	277		340	374	?	566
aglycone of C	277		338	(372)	?	566
aglycone of D	277		340	372	(450)	567

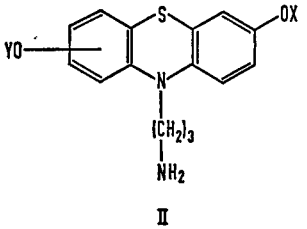
Figures in brackets indicate shoulders on the main peaks.

The electronic spectra of B, C and D in 50% sulphuric acid were similar to that of 3-hydroxypromazine except for a shift of the main peak from 566–7 mμ to 552–4 mμ (see Table 1). Not enough of A was isolated to obtain a satisfactory spectrum, but a peak in the 550 mμ region was observed. After hydrolysis of B, C and D with β-glucuronidase and subsequent extraction, the spectra observed in 50% H_2SO_4 were similar to that of 3-hydroxypromazine except for less intense minor peaks in the 340–370 mμ region. Although this evidence suggests that each of the aglycones of B, C and D are 3-hydroxydesdimethylpromazine, their multiplicity precludes this possibility. Moreover, the glucuronides A, B, C and D change very rapidly to sulphoxides on spectrophotofluorometric examination, whereas 3-methoxypromazine changes slowly to the sulphoxide; more than monohydroxylation of the aromatic nucleus of promazine is thus indicated in these glucuronides.

The structural formulae of B, C and D are then I or II,



I



II

where X = $C_6H_9O_6$ and Y = H,
or X = H and Y = $C_6H_9O_6$

3,7-Dihydroxylation of the promazine nucleus is unlikely because the spectroscopic properties of these glucuronides are completely different from that of 3,7-dimethoxyphenothiazine (see Table 1), which may be considered a suitable reference compound, i.e. the effects exerted in the sulphuric acid test by methoxy and hydroxy groups in phenothiazines are very similar and the addition of an aliphatic side chain on the nuclear nitrogen atom makes only minor differences (Beckett & Curry, 1963).

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Chelsea School of Pharmacy,
Chelsea College of Science and Technology,
Manresa Road,
Chelsea, London, S.W.3
May 28, 1964

A. H. BECKETT
S. H. CURRY
A. G. BOLT

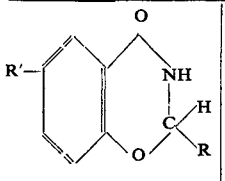
Reference

Beckett, A. H. and Curry, S. H. (1963). *J. Pharm. Pharmacol.*, **15**,246 *T*–253*T*.

Structural requirements for the antiphlogistic activity in some novel derivatives of chlorthenoxazin

SIR,—Kadatz (1957) described the synthesis and the anti-inflammatory properties of chlorthenoxazin, a benzoxazine derivative [2-(2-chloroethyl)-2,3-dihydro-4-oxobenz-1,3-oxazine]. Some years later Baroli, Bottazzi, Ferrari, Garzia, Trabucchi & Vargiu (1963), Ferrari & Garzia (1963), Arrigoni-Martelli (1964) and Arrigoni-Martelli & Conti (1964) described the synthesis and the pharmacological properties of some new derivatives of this compound with various substituents on the nucleus, particularly the 6-amino-derivative (A 350). For the purpose of investigating more deeply the structure-action relationships of this class of compounds, we synthesised a number of new derivatives of chlorthenoxazin (AP 67). The anti-inflammatory activity has been studied on three experimental models of phlogosis of the hind paw of the rats: carrageenin-, dextran-, formalin-oedema (for methods see Arrigoni-Martelli, 1964; Arrigoni-Martelli

TABLE 1. ANTI-INFLAMMATORY ACTIVITY

	Dose mg/kg oral	% Inhibition (± s.d.) of oedema induced by:			Oral LD 50 mg/kg (with confidence limits), rat
		Carrageenin	Dextran	Formalin	
R = CH_2–CH_2–Cl R′ = H (AP 67) Chlorthenoxazin	200	37·0 (±3·2)	22·9 (±4·8)	12·1 (±3·6)	> 2000
R = CH_2–CH_2–Cl R′ = NH_2 (A 350)	195	54·9 (±1·9)	38·8 (±4·2)	27·5 (±4·2)	1958 (1847–2024)
R = Et R′ = H (A 301)	131	33·0 (±2·8)	24·2 (±4·7)	15·6 (±3·9)	1310 (1156–1573)
R = Et R′ = NH_2 (A 302)	189	41·5 (±2·2)	35·3 (±5·1)	18·1 (±3·4)	1890 (1756–1981)
R = Me R′ = H (A 309)	102	25·8 (±2·4)	22·3 (±4·8)	7·5 (±3·1)	1025 (851–1270)
R = Me R′ = NH_2 (A 310)	141	35·8 (±3·1)	32·6 (±5·9)	12·3 (±3·9)	1415 (1282–1593)
R = $CHMe_2$ R′ = H (A 319)	185	20·5 (±2·9)	12·7 (±6·4)	6·4 (±2·1)	1850 (1781–2021)
R = $CHMe_2$ R′ = NH_2 (A 321)	200	26·4 (±2·5)	20·9 (±5·8)	16·6 (±3·9)	> 2000
Phenylbutazone	128	48·2 (±3·1)	29·9 (±4·7)	28·6 (±5·4)	1280 (1156–1325)