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Biotransformations *in vitro* undergone by phenothiazine derivatives in a liver preparation

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Characterisation of the products of biotransformations resulting when a wide range of medicinally used phenothiazine derivatives were incubated with a "microsomal and soluble" fraction of livers from albino Wistar rats, is described. The compounds were chlorpromazine, demethylchlorpromazine, dedimethylchlorpromazine, triflupromazine, promazine, acepromazine, propiomazine, diethazine, promethazine, isopromethazine, ethopropazine, trimeprazine, methotrimeprazine, ethylmemazine, proquamezine, cyamemazine, trifluoperazine, prochlorperazine, thioproperazine, fluphenazine, pecazine, and also the sulphoxides of the first three compounds and of promazine. The predominant reactions were those of dealkylation and of hydroxylation of the aromatic ring. Sulphoxidation did not occur to a significant extent with any of the compounds examined.

APART from the distribution in the tissues, the rate of biotransformation of a foreign compound and the chemical change or changes effected by the organism will inevitably affect the observed pharmacological action of the compound. A comparative study of the changes undergone by pharmacologically diverse phenothiazine derivatives on incubation with a rat liver preparation was undertaken to determine whether the predominant pharmacological action of a given compound might be related to the observed biotransformations.

Experimental

LIVER PREPARATION

The livers of male albino Wistar rats, 200–250 g, were homogenised in the cold with two volumes of potassium chloride solution (1·15% w/v). The homogenate was centrifuged at 9000 rcf. for 15–20 min at 0° to sediment unbroken cells and mitochondria. The supernatant fraction was withdrawn and used (2 ml per reaction mixture) as "microsomal and soluble" fraction. The reaction medium, total volume 8 ml, was as described by Mueller & Miller (1955) but using glucose 6-phosphate in place of the diphosphate and adding the phenothiazine derivative (2·5 μ moles). Five replicate flasks were used for each compound examined. After incubating the reaction vessels with shaking at 37° for 1½ hr, the contents of each set of five flasks were pooled, solid sodium hydroxide (to 3% w/v) was added and the mixture heated on a boiling water-bath for 1 hr. This procedure ensured the subsequent extraction of the phenothiazine derivatives. After the hydrolysis, the extract was transferred to a liquid/liquid extractor and extracted continuously with ether (approximately 125 ml) for at least 2 hr. The ether extract was washed free from alkali and distilled to a low volume from a warm water-bath under reduced pressure. Where storage before further examination was unavoidable, the flask containing the extract was flushed with nitrogen before being sealed and stored in the dark.

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THIN-LAYER CHROMATOGRAPHY

Thin-layer plates, approximately 250 μ thick, were prepared from Aluminium Oxid G (Merck) and from silica gel (Kieselgel, Merck). The alumina plates were oven-dried immediately before use. Solvent development was allowed to proceed to 10–12 cm from the point of sample application, which was 1·5 cm from the lower edge of the plate. After drying, the plates were examined for fluorescence under ultraviolet light.

Solvents. (1) Chloroform containing ethanol (10% by volume) as described previously (Robinson & Beaven, 1964) was used for alumina plates. For silica gel plates, the most useful were (2) chloroform : ethanol : ammonia solution (0·89) 80 : 20 : 1, by volume, and (3) n-propanol : water : acetic acid 85 : 15 : 2, by volume.

The chloroform used for these solvents contained approximately 1% ethanol by volume as a stabiliser. Solvent was replaced after the development of six plates.

Spray reagents. (1) Sulphuric acid 50% v/v; (2) Analar hydrobromic acid (B.D.H., 46–48%); (3) ferric chloride solution (1% w/v in dilute nitric acid); (4) sodium metaperiodate 1% w/v in water (Posner, Culpan & Levine, 1964); (5) ammonium persulphate 5% w/v in water; (6) Gibb's reagent (2,6-dichloro-1,4-benzoquinone-4-chloroimine 1% w/v in ethanol, followed by exposure of the chromatogram to ammonia vapour); (7) sodium nitroprusside-acetaldehyde reagent for secondary amines (Walkenstein & Seifter, 1959).

Rf values. The Rf values for a parent compound and its metabolites quoted in any of the tables were obtained from a single chromatogram. Mean Rf values for chlorpromazine, promazine and triflupromazine were 0·80, 0·70 and 0·80 with standard deviations of 0·06, 0·11 and 0·11 respectively for 30 results obtained on different silica gel plates using solvent (2). The relative positions of the metabolites with respect to the parent compound were constant despite small variations in the absolute Rf values due to minor changes in the experimental conditions.

ABSORPTION SPECTROSCOPY

Spectra were recorded using a Unicam SP 800 spectrophotometer.

The dry residue obtained from evaporation of an ether extract was shaken with 4 ml of sulphuric acid (0·01 N) and the solution filtered through a sintered-glass filter (porosity 3). An aliquot portion was diluted suitably with the acid and the ultraviolet absorption curve was recorded. To the remaining filtrate was added an equal volume of concentrated sulphuric acid and, after cooling, the visible absorption curve was recorded; the ultraviolet absorption curve of this solution was measured after diluting one in ten with sulphuric acid (50% v/v).

Differential spectrophotometry was carried out by replacing the solvent in the reference cuvette with an aqueous solution of the drug or its sulphoxide or a solution of the drug to which an equal volume of concentrated sulphuric acid had been added.

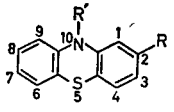
The presence of the sulphoxide of the original drug may be detected by inspection of the ultraviolet absorption curve of an extract in dilute acid solution, in particular by examining the shape of the curve between 270–350 mμ. Differential spectra obtained using both the parent compound and the sulphoxide (when available) confirmed the validity of results obtained by this method. Solutions of the extracts prepared as described above are not contaminated significantly by coenzymes derived from the incubation medium.

Differential measurements on the coloured solutions obtained on adding sulphuric acid to the extracts permitted evaluation of the spectra of some derivatives produced during the biological reaction (see Figs 1 and 3); the sulphides and the corresponding sulphoxides give rise to similar spectra and dealkylation of the 10-dialkylaminoalkyl substituent does not alter the spectrum of the parent compound significantly.

COMPOUNDS

The phenothiazine derivatives were kindly donated by various pharmaceutical companies. Samples which had melting-points corresponding

TABLE 1. FORMULAE OF PHENOTHIAZINE DERIVATIVES EXAMINED



Compound	R	R′
Chlorpromazine	Cl	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$
Demethylchlorpromazine	Cl	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NHMe$
Dedimethylchlorpromazine	Cl	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2$
Triflupromazine	CF_3	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$
Promazine	H	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$
Acepromazine	CO·Me	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$
Propiomazine	CO·Et	$CH_2{\cdot}CH(Me){\cdot}NMe_2$
Diethazine	H	$CH_2{\cdot}CH_2{\cdot}NEt_2$
Promethazine	H	$CH_2{\cdot}CH(Me){\cdot}NMe_2$
Isopromethazine	H	$CH(Me)CH_2{\cdot}NMe_2$
Ethopropazine	H	$CH_2{\cdot}CH(Me){\cdot}NEt_2$
Trimeprazine	H	$CH_2{\cdot}CH(Me){\cdot}CH_2{\cdot}NMe_2$
Methotrimeprazine	O·Me	$CH_2{\cdot}CH(Me){\cdot}CH_2{\cdot}NMe_2$
Ethylmemazine	Et	$CH_2{\cdot}CH(Me){\cdot}CH_2{\cdot}NMe_2$
Proquamezine	H	$CH_2{\cdot}CH(NMe_2){\cdot}CH_2{\cdot}NMe_2$
Cyamemazine	CN	$CH_2{\cdot}CH(Me){\cdot}CH_2{\cdot}NMe_2$
Trifluoperazine	CF_3	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}$N·Me
Prochlorperazine	Cl	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}$N·Me
Thioproperazine	$SO_2{\cdot}NMe_2$	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}$N·Me
Fluphenazine	CF_3	$CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}$N·$CH_2{\cdot}CH_2OH$
Pecazine	H	CH_2–N·Me

with those given in the literature and which migrated as single substances on thin-layer chromatoplates were used without further purification. The compounds examined, with their formulae, are listed in Table 1.

Results and discussion

CHLORPROMAZINE AND ITS DEMETHYLATED AND SULPHOXIDISED ANALOGUES

Chloropromazine. The results using chlorpromazine were similar to those described previously using the whole liver homogenate (Robinson & Beaven, 1964) except that hydroxylated demethylchlorpromazine and traces of chlorpromazine sulphoxide were detected as well. There was no evidence of oxidation at the amino-nitrogen atom.

TABLE 2. CHARACTERISTICS OF CHLORPROMAZINE METABOLITES, RECOVERED FROM THE INCUBATION MEDIUM, ON SILICA GEL CHROMATOPLATES

Compounds	Rf on silica gel plates	
	Solvent (2)	Solvent (3)
Chlorpromazine	0·80	0·30
Chlorpromazine sulphoxide	0·62	0·08
Demethylchlorpromazine	0·53	0·45
Hydroxychlorpromazine	0·47	0·23
Demethylhydroxychlorpromazine	0·15	0·03
Dedimethylchlorpromazine	*	0·60

* Runs with demethylchlorpromazine in this solvent.

Thin-layer chromatographic characteristics of the metabolites are given in Table 2. Demethylchlorpromazine gave a blue colour with the sodium nitroprusside-acetaldehyde reagent, as did the metabolite referred to as demethylhydroxychlorpromazine. The two hydroxylated metabolites consistently gave a purple colour with sulphuric acid, hydrobromic acid and ferric chloride, and an immediate blue colour with Gibb's reagent. Most of the phenothiazine compounds examined gave a blue-grey colour with Gibb's reagent and with sodium nitroprusside-acetaldehyde on standing, presumably due to an oxidising reaction. The colour produced by the hydroxylated compounds with sodium periodate was usually a brick red, as is given by 7-hydroxychlorpromazine, but in some of the extracts examined a definite pale emerald-green colour was obtained. The ammonium persulphate reagent gave similar colours and was preferred because the colours were more intense and more persistent. The green colour with these reagents was seen only on plates developed in a non-acidic solvent; the presence of traces of acid resulted in a lavender-purple colour.

The ultraviolet absorption characteristics of the extract in dilute acid solution were not remarkable and easily resolved by differential measurements into sulphide (λ_{max} 255 and 305 mμ), which predominated, and a trace of sulphoxide (λ_{max} 239, 275, 290 and 342 mμ).

Differential visible absorption spectra in strong acid solution indicated the presence of a hydroxy-compound (λ_{max} 570 mμ) as well as chlorpromazine (λ_{max} 530 mμ). The ultraviolet absorption peaks of dilutions

(with 50% v/v) sulphuric acid of the coloured solutions of the extracts were at 218, 270 and 277 mμ; chlorpromazine has peaks at 213, 268 and 277·5 mμ under the same conditions.

In the current work a greater proportion of the initial chlorpromazine underwent biotransformation reactions than was found previously (Robinson & Beaven, 1964). This was partly due to an increase in the incubation time but mainly due to the use of a fraction of the liver rather than the whole homogenate, thus minimising the non-specific interactions with proteins (Gabay & Harris, 1965) other than those associated with enzymes involved in the biotransformation reactions. The contamination, assessed spectrophotometrically, of the solutions with coenzymes was negligible.

TABLE 3. SUMMARY OF CONCLUSIONS OF THE BIOTRANSFORMATIONS FOUND FOR CHLORPROMAZINE ANALOGUES

Compound incubated	Transformation products found*
Chlorpromazine	Chlorpromazine sulphoxide (trace) Demethylchlorpromazine Dedimethylchlorpromazine Hydroxychlorpromazine Demethylhydroxychlorpromazine
Chlorpromazine sulphoxide	Chlorpromazine (trace)
Demethylchlorpromazine	Demethylchlorpromazine sulphoxide Demethylhydroxychlorpromazine
Demethylchlorpromazine sulphoxide Dedimethylchlorpromazine Dedimethylchlorpromazine sulphoxide	None

* In each case the original compound incubated was also detected in the preparation at the end of the incubation.

Since no chlorpromazine sulphoxide could be detected when a control chlorpromazine solution was carried through the entire experimental procedure, omitting only the addition of the tissue preparation to the incubation mixture, it seems unlikely that the chlorpromazine sulphoxide found after incubation was an artifact. Similar stability was found with the other compounds included in this series of experiments.

The hydroxylated chlorpromazine derivative which gave the unusual green colour with periodate and persulphate reagents was obtained on several occasions. In other experiments, a substance having similar chromatographic characteristics was obtained; this gave a reddish-brown colour with these reagents. The spectral absorption properties of the extracts containing these different metabolites were similar although minor differences could have been masked by the predominance of non-hydroxylated compounds. It is considered that these two metabolites may differ only in the position of the hydroxyl group in the phenothiazine ring system.

Chlorpromazine analogues. Thin-layer chromatographic data for the extracts obtained after incubation of the demethylated and sulphoxidised analogues of chlorpromazine with the liver preparation, together with the absorption spectral data for the dilute and strong sulphuric acid solutions of the extracts, yielded the conclusions summarised in Table 3.

These results are relevant to the current polemics concerning the mechanism of biological hydroxylation of phenothiazine derivatives. Under the appropriate chemical conditions some phenothiazine-5-oxides rearrange to the corresponding 3-hydroxyphenothiazine (see Craig & Tate, 1961), which has caused speculation that biological sulphoxidation could precede rearrangement to a ring hydroxy-derivative; the latter reaction could be effected either biologically or non-biologically. Alternatively, sulphoxidation and hydroxylation could be separate biological reactions.

The biotransformation reactions seen in the present work for chlorpromazine and demethylchlorpromazine include hydroxylation and, to a lesser extent, sulphoxidation; comparatively more of the initial chlorpromazine was metabolised (A. E. Robinson and S. E. Selim, to be published). Since the three sulphoxides (except for a trace of a reduction product of chlorpromazine sulphoxide) were recovered unchanged from the incubation mixtures, an enzyme-catalysed rearrangement to a hydroxy-compound appears to be unlikely. It is possible that if the enzyme is located within the hepatic microsomes the physical characteristics, including the more nearly planar shape and the partition coefficient may prevent the sulphoxide gaining access to the enzyme site(s).

TABLE 4. THIN-LAYER CHROMATOGRAPHIC CHARACTERISTICS OF EXTRACTS FROM INCUBATION MEDIA CONTAINING TRIFLUPROMAZINE

Solvent 1*	Solvent 2**	Solvent 3**	Fluorescence	Colours given with reagents: 1 & 2	3	4	5	6	7	Identity concluded
0·84	0·84	0·28†	Green	O	O	pO	O	—	—	Triflupromazine
0·80	0·70	0·10	Blue	O	—	O	O	—	—	Triflupromazine sulphoxide
0·52	0·60	0·50	Green	O	O	O	O	—	B	Demethyltriflupromazine
0·30	0·54	0·35	Green	Pu	Pu	Pi/Pu	Pi/Pu	G	—	Hydroxytriflupromazine
0·02	0·23	0·62	Green	Pu	Pu	Pi/Pu	Pi/Pu	G	B	Demethylhydroxytriflupromazine

* Alumina chromatoplate. ** Silica gel chromatoplate. † Ran immediately behind hydroxy triflupromazine. Colour code: O = Orange; B = Blue; Pu = Purple; Pi = Pink; G = Grey-green

Triflupromazine. The chromatographic and spectral absorption properties of the extracts obtained after incubation of triflupromazine with the liver fraction are summarised in Tables 4 and 5. Fig. 1 shows the visible absorption spectra obtained using (*a*) normal reagent blank (curve 1), (*b*) triflupromazine (curve 2) and (*c*) 7-methoxytriflupromazine (curve 3) in the reference beam. Clearly, the extract contains two absorbing components: unchanged triflupromazine and a hydroxy-derivative (cf. Table 5), and possibly a third. In most instances, the absorption peak of the hydroxylated triflupromazine was at about 570 mμ; this metabolite appears to be 7-hydroxytriflupromazine. However, a metabolite having the same Rf value in systems 1 and 2 and giving the same colour reactions with all reagents except periodate and persulphate was recovered from a developed thin-layer chromatoplate and gave a well-defined spectrum in strong sulphuric acid with peaks at 217–8, 270,

TABLE 5. LIGHT ABSORBING PROPERTIES OF SOLUTIONS OF TRIFLUPROMAZINE, EXTRACTS FROM INCUBATION MEDIA TO WHICH TRIFLUPROMAZINE WAS ADDED, AND REFERENCE COMPOUNDS

Compound	Ultraviolet absorption peaks* in 0·01 N sulphuric acid solution	Comments	Ultraviolet and visible absorption peaks* in 50% v/v sulphuric acid solution	Comments
Triflupromazine	(235) 256 306		207 (250) 275 505	Reference compound
Triflupromazine sulphoxide.. ..	237 274, 298 340		207 (250) 275 505	Reference compound
Extract from incubation with triflupromazine	(235) 256 306	Slight distortion at 235–40 and 270–85	210 276 520 and 568 505, 568	Unresolved Resolved†
Extract from incubation with triflupromazine and proadifen	256 304		206 276 504–5	
7-Methoxytriflupromazine	(240) 258 308		225 282 (370) (450) 573	Reference compound
7-Methoxy-2-trifluoromethyl-phenothiazine	217 (240) 262 314	In ethanol	222, 249 279 348, 376 436 558	Reference compound
8-Methoxy-2-trifluoromethyl-phenothiazine	235, 262 303 (335)	In ethanol	226 280 350 (460) 559	Reference compound

* Wavelengths in mμ. Those in brackets refer to shoulders on the curves. † see text.

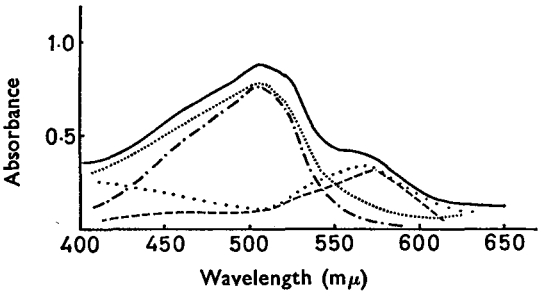


FIG. 1. Visible absorption curves in 50% v/v sulphuric acid for an extract derived from incubation of the liver fraction with triflupromazine. 1. ——— Extract against 50% v/v sulphuric acid. 2. . . . . Extract against triflupromazine in 50% v/v sulphuric acid. 3. - - - - - Extract against 7-methoxytriflupromazine in 50% v/v sulphuric acid. 4. — · — · — Solution of triflupromazine in 50% v/v sulphuric acid used as reference for 2 against 50% v/v sulphuric acid. 5. — — — Solution of 7-methoxytriflupromazine in 50% v/v sulphuric acid used as reference for 3 against 50% v/v sulphuric acid.

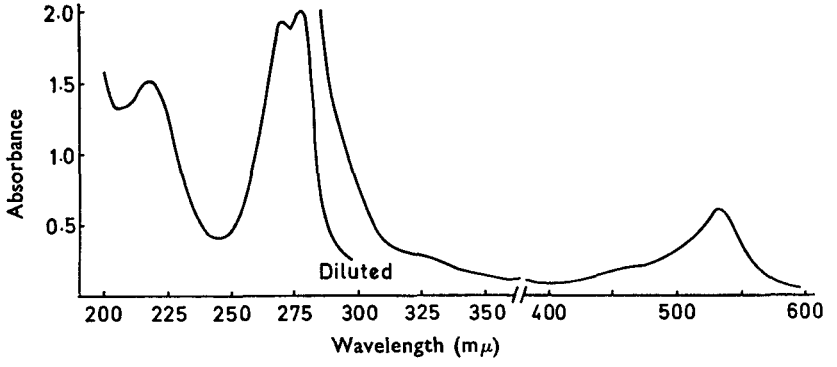


FIG. 2. Absorption spectrum of a hydroxylated triflupromazine derivative in 50% v/v sulphuric acid recovered from a thin-layer chromatoplate.

277·5, 350 (shoulder), 376 and 533 mμ (see Fig. 2). This metabolite is thought to be a position isomer, probably 3-hydroxytriflupromazine or, possibly, 8-hydroxytriflupromazine (see Table 5 and cf. chlorpromazine).

Qualitatively similar results were obtained when the initial concentration of triflupromazine in the incubation mixtures was varied between 1 and 5 μmoles per flask.

Addition of proadifen hydrochloride (SKF 525-A; 2-diethylaminoethyl-αα-diphenylvalerate hydrochloride) to the incubation mixture containing triflupromazine at a molar ratio of between 1 to 6 and 1 to 2 prevented hydroxylation of the compound; demethylation was unaffected and no greater proportion of sulphoxide was noted. This inhibition of a detoxication reaction would explain the enhanced or prolonged pharmacological action in animals treated with the inhibitor. The hydroxylated phenothiazine derivatives as a whole seem to be less active pharmacologically than the parent compounds.

OTHER PROMAZINE DERIVATIVES

The remaining compounds of this group which were examined included promazine and the corresponding sulphoxide, acepromazine, and propiomazine.

TABLE 6. THIN-LAYER (SILICA GEL) CHROMATOGRAPHIC CHARACTERISTICS OF EXTRACTS FROM INCUBATION MEDIA CONTAINING PROMAZINE AND FOR REFERENCE COMPOUNDS

	Rf		Reagents			
	Solvent 2	Solvent 3	1 & 2	4	5	Compound
Extract	0·75	0·19	O/Pi	pPi	O/Pi	Promazine
	0·46	0·24	Pu	Pi/Br	Pu/Br	3-Hydroxypromazine
	0·43	0·37	O/Pi	Pi/Br	Pi/Br	Demethylpromazine
	0·16	0·4	Pu	Pi/Br	Pu/Br	Demethyl-3-hydroxy-promazine
Reference compounds	0·74	0·22	O/Pi	pPi	O/Pi	Promazine
				L	L	1-Hydroxypromazine
	0·4	0·24	Pu	T	T	2-Hydroxypromazine
	0·45	0·25	Pu	R/Pu	G/Br	3-Hydroxypromazine
	0·62	0·24	pPi/Br	Pi/R	Cr	4-Hydroxypromazine
	0·65		O/Pi	pPi	O/Pi	Promazine sulphoxide

Colour code: O = Orange; B = Blue; Pu = Purple; Pi = Pink; G = Grey-green; Br = Brown; Cr = Crimson; L = Lavender; R = Red; T = Turquoise.

Promazine. The thin-layer chromatographic results, which are summarised in Table 6, confirm and extend the previous results with the whole liver homogenate system (Robinson & Beaven, 1964). The ultraviolet absorption spectrum of the dilute acid solution of the extract corresponded exactly with that of promazine: there was no indication of sulphoxidation. The visible absorption spectrum in strong acid solution showed an absorption peak at 519 mμ together with a shoulder at 560–580 mμ; difference measurements with promazine in strong acid in the reference beam gave the absorption peak of the hydroxylated derivative as 566–8 mμ. 3-Hydroxypromazine has an absorption peak at 566–8 mμ under these conditions.

Promazine sulphoxide was recovered unchanged after incubation with the liver preparation.

Acepromazine and propiomazine. The data obtained for these compounds are included in Tables 7 and 8. Both derivatives underwent hydroxylation but demethylation was detected only with acepromazine.

MISCELLANEOUS ALKYLAMINOALKYLPHENOTHIAZINE DERIVATIVES

Various compounds were investigated to determine whether slight differences in chemical structure influenced the pathways of biotransformation. The data obtained are summarised in Tables 7 and 8.

The length of the alkyl chain attached to position 10 of the phenothiazine nucleus is reduced to two carbon atoms in diethazine. A branched methyl group is present on the two-carbon chain in promethazine and isopromethazine while ethopropazine is the diethylamino-analogue of promethazine.

TABLE 7. CHARACTERISTICS OF METABOLITES EXTRACTED FROM INCUBATION MIXTURES (SILICA GEL PLATES AND SOLVENT SYSTEM 2)

Compound added to incubation medium	Metabolites recovered from incubation mixture		
	Unchanged compound	Dealkylated analogues*	Hydroxylated analogues**
Acepromazine[1]	0·78 Orange†	0·42 Orange†	0·48 Orange L (1, 2, 3)‡ O (4) 0·22
Propiomazine[2]	0·92 Orange†		0·45 Orange† L (1, 2, 3) R (4)
Diethazine	0·89 Blue†	0·75 Blue†	0·62 Dark blue† Pu (4) O (5) 0·47 blue† Y/Br (4 & 5)
Promethazine	0·88 G (4) I (5)	0·76 G (4 & 5)	0·67 L (1, 2, 3) R/Pu (4 & 5) 0·60 L (1, 2, 3) G/Y (4) Y (5)
Isopromethazine[3]	0·94 G (4) B/G (5)	0·88	0·80 B (1, 2, 3) R/Br (4 & 5) 0·7 B (1, 2, 3) Y (4 & 5)
Ethopropazine[4]	0·80 Blue†	0·66 Blue†	0·38 Blue† L (1, 2, 3) Pi with 4
Trimeprazine	0·89 Pi (1, 2, 3)	0·57	0·63 O (4) Pi (5) 0·23 O (4) Pi (5)
Methotrimeprazine ..	0·89 B/Pu (1, 2, 3) Pi (4)	0·63, 0·23 } Pu (5)	0·57, 0·18 } Y (5)
Ethylmemazine	0·82 Pi (1, 2, 3)	0·53	0·58 L (1, 2, 3) 0·36 Pu (5)
Proquamezine	0·63 Pi (1, 2, 3) G (5)	0·43	0·35 Pi (4) G/Pi (5)
Cyamemazine	0·78 Yellow/green† O (1, 2, 3)	0·42	0·49 Pi (4) Y/Br (5)
Trifluoperazine	0·80 Green† O (1, 2, 3, 5)	0·39 Green†	0·51 L (1, 2, 3) 0·14 Pi/O (4) L (5)
Prochlorperazine	0·72	0·24	0·38 Li (1, 2, 3) Pi/Br (4)
Thioproperazine	0·59 Green†	0·32	0·25 L (1, 2, 3, 4)
Fluphenazine	0·68 Green† O (1, 2, 3) B/G (5)	(0·27—see text)	Pu (1, 2, 3) O/Br (4, 5) 0·32
Pecazine	0·86 O/Pi (1, 2, 3) Pi/Br (5)	0·28	0·52 Pu (1, 2, 3) O/Br (4) Pi (5)

* Positive reaction with reagent 7. ** Positive reaction with Gibb's reagent, etc. † Fluorescence under ultraviolet light. ‡Spray reagents—see text. [1–4]Sulphoxides: positive reaction with sulphuric acid but negative with ferric chloride reagents; [1]0·56 blue (trace)†; [2]0·52 blue (trace)†; [3]0·76 (trace)†; [4]0·13 (trace)†. For colour code see Table 6.

Dealkylation and hydroxylation, separately, were undergone by each of these compounds. The hydroxylated analogues all gave characteristic colours with the sodium periodate and ammonium persulphate reagents (Table 7) and possessed absorption peaks in strong sulphuric acid solution in the visible region at wavelengths longer than those of the parent

TABLE 8. LIGHT ABSORBING PROPERTIES OF THE DRUGS AND SOLUTIONS OF EXTRACTS OBTAINED FROM INCUBATION MIXTURES

Compound added to incubation medium	λ_{max} in mμ of ultraviolet absorption peaks in 0·01 N sulphuric acid solution	λ_{max} in mμ absorption peaks in 50% v/v sulphuric acid solution
Acepromazine	205 243 278	212 235 (252, 290) 515
Extract	207 243 278	(230) 254, 290 522 and 570 unresolved 515 and 572 resolved*
Propiomazine ..	204 241	204 241 289 522
Extract	204 241 (275) 342, 358 (ill-defined)	239 277 528 and 580 unresolved 522 and 583 resolved*
Diethazine	250 300	211 266 274 517
Extract	(235) 247 295 Trace of sulphoxide	214 266 275 518 and shoulder 560–80 518 and 572 resolved*
Promethazine ..	202 250 298	211 267 275 (440) 518–20
Extract	204 249 296–8	212 267 276 (438) 523 and 572 unresolved 518–20 and 573 resolved*
Isopromethazine ..	202 252 303	213 257 (270–5) 520–5
Extract	(235) 251 295 Trace of sulphoxide	212 262 273 Wide band 520–580 520, 565–70, 600 resolved*
Ethopropazine ..	249 302	211 266–271 (broad) 519
Extract	248 295 Trace of sulphoxide	213 268 276 (372, 440) 520 and 560 519 and 570 resolved*
Trimeprazine ..	252 300	213 268 275 517
Extract	252 298	212 268 275 (450) 518 and shoulder 570 516 and 576 resolved*
Methotrimeprazine	250 300	223 281 572
Extract	249 298	223 (253) 282 572
Ethylmemazine ..	251 300	211 266 276 531
Extract	250 298	218 (270) 278 534 distorted 531 and 579 resolved*
Proquamezine ..	253 301	250 275 520
Extract	250 300	250 274, 440 519 and shoulder 570 516 and 570–575 resolved*
Cyamemazine ..	232 268	(235) (250) 286 513
Extract	235 265	516 distorted 513 and 570 resolved*
Prochlorperazine ..	254 303	212 269 277 530
Extract	254 303	216 269 277 530 and 575 (weak)
Thioproperazine ..	265 315	210 (251) 282 518 distorted
Extract	263 315	210 251 283 503 and 570 resolved*
Fluphenazine ..	256 306	210 (250) 275 503 distorted
Extract	256 306	210 (250) 275 503 and 565 resolved*
Pecazine	253 303	213 267 274 516
Extract	253 300	213 267 274 516 and 563 resolved*

* Wavelengths are those obtained from differential measurements.

compounds (Table 8, and cf. Fig. 3). The value of using both of the aforementioned reagents for the location and characterisation of hydroxy-derivatives is seen particularly in the case of promethazine; both of the 'hydroxy' spots on the chromatoplates gave the same colours with the acidic reagents (1, 2 and 3, p. 20) but differed in their reactions with periodate (4) and persulphate (5). One of the two compounds reacted differently with each of the reagents. Similarly with isopromethazine, differentiation of the hydroxy-derivatives was possible only with the periodate and persulphate reagents on neutral or alkaline chromatoplates; if acid was present in the developing solvent or in the spray reagent, as in the persulphate reagent used by Goldenberg & co-workers (1964), the colours seen with the acidic reagents were obtained. Although two hydroxy-derivatives were apparent on thin-layer plates of extracts from promethazine incubation mixtures, only one was detected spectrophotometrically (λ_{max} 573 mμ in 50% v/v sulphuric acid), perhaps implying that the compounds differ in the nature of the alkyl chain attached to the ring nitrogen atom. However, the two hydroxy-derivatives present in the isopromethazine extracts did give two different absorption peaks (λ_{max} 565–570 and 600 mμ) in strong acid solution suggesting that, in this case, the difference involves the chromophore directly.

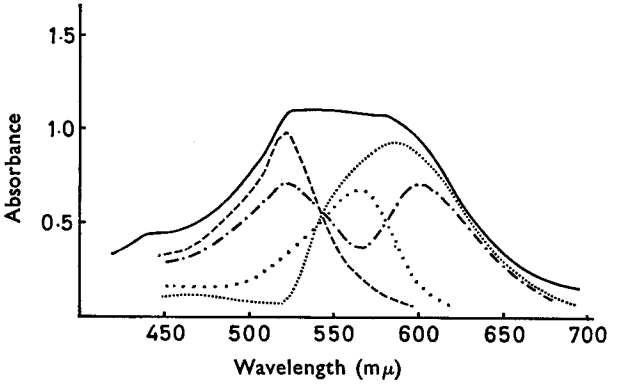


FIG. 3. Visible absorption curves in 50% v/v sulphuric acid for an extract derived from incubation of the liver fraction with isopromethazine. 1. ——— Extract against the acid. 2. - - - - - Extract against isopromethazine in 50% v/v sulphuric acid. 3. — — — Solution of isopromethazine in 50% v/v sulphuric acid used as reference for 2 against 50% v/v sulphuric acid. 4. — · — · — Extract against 3-hydroxypromazine in 50% v/v sulphuric acid. 5. . . . . Solution of 3-hydroxy-promazine in 50% v/v sulphuric acid used as reference for 4 against 50% v/v sulphuric acid.

The wavelengths of the absorption peaks of the hydroxylated analogues of diethazine, promethazine, ethopropazine and one of the isopromethazine derivatives in strong acid solution are as expected in the absence of other ring substituents. In all cases where hydroxylation had occurred, observation of the sequence of colour changes seen on addition of concentrated sulphuric acid to the dilute acid solution of the extract

was informative. Usually, when the acid was added dropwise and with mixing, the purple colour associated with the hydroxy-compound developed first and became pinker or more orange, depending upon the parent compound, as the concentration of the acid was increased. It was sometimes possible to obtain a spectrum for the hydroxy-compound before that of the parent compound was fully developed, but the stability of the spectra for the former was poor compared with those for the latter compounds.

Despite the relative ease of oxidation of these compounds, sulphoxides were either totally absent or were present in trace amounts only.

The effect of introducing an additional group to the 3-dimethylaminopropyl substituent present in promazine was also studied. These compounds included trimeprazine, methotrimeprazine, ethylmemazine, proquamezine and cyamemazine.

Demethylation was undergone by each of these compounds although, with proquamezine, it was impossible to determine at which dimethylamino-group the reaction occurred. The hydroxylated analogues again showed absorption peaks in strong acid solution at wavelengths longer than those of the parent compounds. None of the compounds in this group yielded a sulphoxide.

SOME 10-HETEROCYCLIC-SUBSTITUTED PHENOTHIAZINE DERIVATIVES

The phenothiazine derivatives containing a heterocyclic ring as part of the substituent in position 10 examined were trifluoperazine, prochlorperazine, thioproperazine, fluphenazine and pecazine. With each of the compounds containing the 4-methylpiperazinyl group, a spot on the developed chromatoplate was obtained which gave a positive reaction with the nitroprusside-acetaldehyde reaction for secondary amines. A secondary nitrogen group could arise either from demethylation of the methyl piperazinyl group or by opening of the piperazine ring. Similarly, pecazine also formed a substance which reacted as a secondary amine. Fluphenazine, which has a hydroxyethyl group on the piperazine ring gave a similarly placed spot on the developed chromatoplate in solvent 2 but it did not react with the reagent.

Each compound in this group was hydroxylated on the phenothiazine ring. However, the reduced contribution to the observed spectrum in strong acid solution in the visible region, compared with the corresponding promazine derivatives, suggested that hydroxylation occurs less readily with these compounds which, consequently, are effective at lower dose levels. The sulphoxides, which all show fluorescence at a shorter wavelength than the parent compounds when viewed under ultraviolet light, were not detected in the extracts and it seems unlikely that biological sulphoxidation precedes hydroxylation in these systems. Thus, all of the phenothiazine derivatives studied were susceptible to the same pathways of hepatic biotransformation and there is no obvious correlation between chemical structure and pharmacological activity on this basis. The pharmacological potency of each compound would, therefore,

appear to depend on the quantitative relationships between the tissue levels of the drugs and their rate of biotransformation and subsequent elimination.

The type of action effected by any particular compound may be related to the actual distribution of the compound and the possible metabolic products formed in, or transported to, the various tissues. This aspect is being studied further.

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