J. Pharm. Pharmacol. 1979, 31: 512–516 Received October 10, 1978

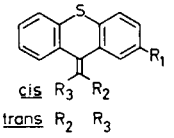
A high performance liquid chromatographic assay of *cis*- and *trans*- isomers of tricyclic neuroleptic drugs

A. LI WAN PO AND W. J. IRWIN*

Department of Pharmacy, The University of Aston, Gosta Green, Birmingham B4 7ET, U.K.

Tricyclic neuroleptics based upon the thioxanthene nucleus exhibit geometrical isomerism and the major pharmacological activity resides in the Z-component. An h.p.l.c. procedure which enables the separation detection and quantification of these isomers is described. The method is applicable to the analysis of flupenthixol, clopenthixol, chlorprothixene, doxepin and dothiepin. Measurement of the isomer-ratios in various samples of flupenthixol has shown that small batch to batch variations are apparent. The determination of the isomer-ratio in formulations has been shown to rely upon the complete extraction of the medicament. This is due to the differential release of the components from the tablet matrix with the *cis*-isomer being favoured. There is little difference observed between the adsorption isotherms of the two components (onto charcoal) but in competition experiments differential adsorption may be demonstrated. This has clear implications for the pharmacokinetics of these drugs.

The presence of the exocyclic olefin residue and a non-symmetrical tricyclic structure allows the neuroleptic thioxanthenes flupenthixol (I), clopenthixol (II), chlorprothixene (III), and thiothixene (VI) to exist as either a *cis*- (*Z*, α) or as a *trans*- (*E*, β) geometrical isomer. Doxepin (IV) and dothiepin (V) have structural resemblance to these thioxanthenes. The original literature on all of these compounds reports the equilibrium composition isomers, which typically range from doxepin [15% *cis*] (Hobbs 1969) through thiothixene [37% *cis*] (Muren & Bloom 1970) to flupenthixol [ca 50% cis] (Møller Nielsen et al 1973). Chlorprothixene (III) is described as the *Z* isomer (Martindale 1977a), dothiepin is described as mainly the *trans*-isomer (Martindale 1977b) and doxepin consists of 15% *Z* (*cis*) isomer (Merck Index 1976). The *cis*- isomer of doxepin is more active than the *trans*- (Bloom & Tretter 1964). In the thioxanthene series the available evidence suggests that most of the neuroleptic activity of the drug resides in the *cis*-isomer. This has been shown in vitro by the inhibition of a dopamine-stimulated increase in adenylate cyclase activity in rat brain homogenates (Horn et al 1975; Miller et al 1974) and in competitive binding studies using, for example, the competition against haloperidol for the dopamine receptor (Enna et al 1976; Burt et al 1976). Other studies confirm these results (Seeman & Lee 1975; Bouillin et al 1975; Meltzer et al 1977). Results in vivo parallel this activity difference between isomers and show very low activity for the *trans*-isomer (Møller Nielsen et al 1973). Although differences have not been demonstrated conclusively in man (Crow et al 1977) there seems little doubt that a significant difference in activity between *cis*- and *trans*-isomers is to be expected (Enna et al 1977).



cis R_3 R_2

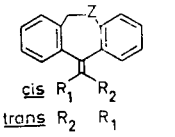
trans R_2 R_3

Flupenthixol (I) R_1=CF_3 R_2=CH_2-CH_2-CH_2-N⟨⟩N-CH_2-CH_2OH R_3=H

Clopenthixol (II) R_1=Cl R_2=CH_2-CH_2-CH_2-N⟨⟩N-CH_2-CH_2OH R_3=H

Chlorprothixene(III) R_1=Cl R_2=CH_2-CH_2-CH_2-$N(CH_3)_2$ R_3=H

Thiothixene (VI) R_1=$SO_2N(CH_3)_2$ R_2=CH_2-CH_2-CH_2-N⟨⟩N-CH_3 R_3=H



cis R_1 R_2

trans R_2 R_1

Doxepin (IV) R_1=H R_2=CH_2-CH_2-CH_2-$N(CH_3)_2$ Z=O

Dothiepin (V) R_1=H R_2=CH_2-CH_2-CH_2-$N(CH_3)_2$ Z=S

Studies on the distribution and metabolism of these compounds have paid little attention to possible variations in the fate of the isomers. Doxepin isomer ratios before and after administration were equivalent (Hobbs 1969). Thioxanthene studies have concentrated on monitoring the distribution of radioactive-labelled drugs and the identification of metabolites by t.l.c. comparison with authentic

* Correspondence.

samples (Jørgensen & Gottfries 1972; Jørgensen et al 1969, 1971; Raaflaub 1967; Weissman et al 1974).

The inadequacies of methods of assay have not encouraged work with the isomers. Flupenthixol, for example, has been assayed by infrared spectroscopy (Uda et al 1970), a technique fraught with experimental difficulties, and although chromatographic analysis has been widely used (Dell et al 1970; 1971; de Leenheer 1973) the separation and quantification of isomers is time-consuming and tedious (Muren & Bloom 1970). H.p.l.c. identification of doxepin (Vandemark et al 1978) makes no mention of the isomers and an h.p.l.c. assay of thiothixene (Wong et al 1976) did not separate isomers.

We have devised a method for the rapid analysis of the *cis*- and *trans*-isomers of tricyclic neuroleptic drugs and have used it to assess the isomer ratios of various batches of pure and formulated flupenthixol.

MATERIALS AND METHODS

Apparatus

Analyses were made using a high-pressure liquid chromatograph constructed from an Altex 100A constant flow solvent-metering pump, a Rheodyne 7120 injector fitted with a 20 μl loop, and a Cecil 212 variable wavelength uv monitor, equipped with an 8 μl flow-cell and operated at 260 nm with a sensitivity of 0·5 AUFS. For chromatography, a 25 cm × 4·6 mm i.d. column of Spherisorb (5 μm spherical, totally porous silica) was used with a mobile phase consisting of ethyl acetate, methanol, 3% w/v ammonia (85:15:1 v/v) delivered at a rate of 1 ml min^{-1} under a pressure of 70 bar.

Standard solutions of *cis*- and *trans*- flupenthixol dihydrochloride were prepared as follows: separate aqueous solutions containing *cis*- or *trans*-flupenthixol dihydrochloride from 0·06–0·3 mg ml^{-1} were prepared. Each solution was added to an aqueous solution of promazine hydrochloride (0·25 mg ml^{-1}) (internal standard), and the whole was basified with aqueous sodium hydroxide (5M, 1 ml). Ethyl acetate (2 ml) was added, the mixture thoroughly agitated, the phases allowed to separate and the supernatant (20 μl) then injected onto the h.p.l.c. column. The ratio of the peak heights of flupenthixol and promazine was used to calculate calibration lines for the *cis*- and *trans* isomers. The use of standard solutions each containing both isomers of flupenthixol dihydrochloride [total concentration 0·6 mg ml^{-1} in varying proportions, or with varying concentrations and equal proportions] also enabled linear calibration lines to be obtained but in a much shorter time. By varying the amount of internal standard, the method could be used for concentrations of flupenthixol from 1·8 to 0·001 mg ml^{-1} without modification.

Batch samples of the drugs were assayed by the method after the preparation of solutions containing 0·3–0·6 mg ml^{-1} of the hydrochloride salts. Tablets of flupenthixol [Depixol (3 mg) Fluanxol (0·5 mg)] were assayed after extraction with 5 or 10 ml of water or hydrochloric acid (0·1, 0·5, 1 M). The suspensions were agitated ultrasonically for 90 min, centrifuged and 1 ml of the supernatant assayed as before. The adsorption of *cis*- and *trans*-flupenthixol dihydrochloride on activated charcoal (10 mg) was studied by equilibrating 20 ml of aqueous solutions of each isomer or a mixture of isomers, each containing 1–10 × 10^{-4} M drug with the adsorbent (charcoal). After equilibration the suspensions were centrifuged and the residual flupenthixol was determined by the measurement of the absorbance at 230 nm. For h.p.l.c. assay, 10 ml volumes of solutions of flupenthixol (combined *Z* and *E* isomers) dihydrochloride 3 × 10^{-3} M (1·521 mg ml^{-1}) in (a) water and (b) M HCl were treated with activated charcoal (50 mg) and 1 ml of the supernatant was assayed as described above. All solutions were kept in the dark before analysis.

RESULTS AND DISCUSSION

Typical h.p.l.c. traces are reproduced in Fig. 1 and the chromatographic parameters are recorded in Table 1 which also includes other tricyclic drugs so that our system may be related to published analyses (Vandemark et al 1978). These data illustrate the optimum analysis conditions for time and resolution of the *cis-trans* isomers of the compounds. Ammonia was included as a modifier in the system to prevent excessive broadening of the eluted bases by interaction with the acidic silica packing. A decrease in both analysis time and resolution was caused by increases in the methanol or ammonia content of the mobile phase. Thiothixene isomers did not separate under these conditions. A single peak with a retention time of 20 min was observed. The sulphonamido function at the 3-position of the thioxanthene causes a marked increase in the polarity of this molecule which results in stronger interactions with the silica stationary phase overriding the weaker stereochemical effects.

In all other cases the *cis*-isomers were eluted before the *trans*-analogue, with a resolution typic-

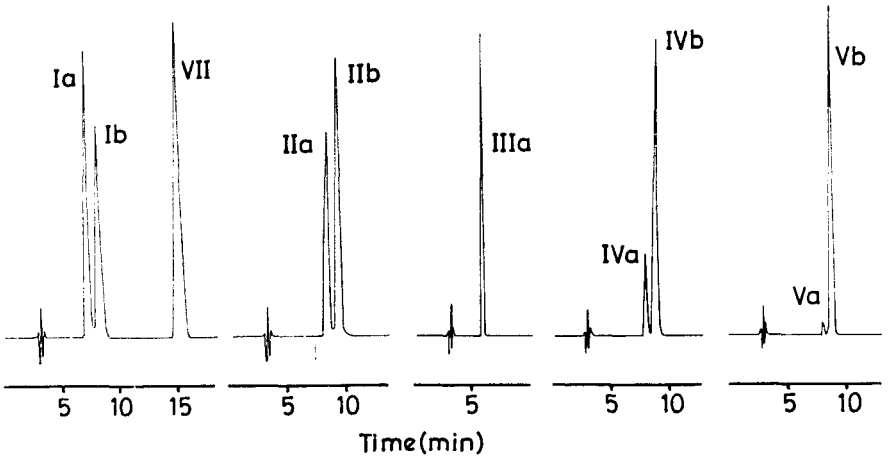


FIG. 1. H.p.l.c. separation of (a) *cis*- and (b) *trans*-isomers of tricyclic neuroleptics: flupenthixol (I), clopenthixol (II), chlorprothixene (III), doxepin (IV), dothiepin (V) and promazine (VII).

ally of 1–2. This retention difference may be due to differential interaction with adsorbed mobile phase or to a decreased polarity, indicated by dipole moment measurements (Petersen & Møller Nielsen 1964), of the more active *cis*-isomer* resulting in a weaker adsorption on to the silica column.

To assess the application of this system to the quantification of isomers, the analysis of samples of flupenthixol was studied. Calibration lines over a concentration range suitable for the determination of flupenthixol tablets were constructed and the equations for the slopes are: *trans*, $y = 294{\cdot}8\ (\pm 4{\cdot}2)x + 0{\cdot}8$ and *cis*, $y = 415{\cdot}8\ (\pm 5{\cdot}3)x - 0{\cdot}9$ ($n = 11$). The differences in slope is mainly due to the greater elution volume of the *trans*-isomer although small differences in absorbance may also contribute to this effect (Muren & Bloom 1970). By constructing appropriate calibration lines the method could be applied to solutions ranging from 1·8 mg ml^{-1} down to 1 μg ml^{-1} without modification, although at lower levels of detection longer column equilibration times were necessary to achieve a steady base line.

Table 1. Retention data for *cis*- and *trans*- isomers.

Drug	Rt Min *cis*	Rt Min *trans*	Capacity ratio[1] K′ *cis*	Capacity ratio[1] K′ *trans*	Resolution[2] *R*
flupenthixol (1)	7·09	8·05	1·44	1·78	1·28
clopenthixol (2)	8·02	8·89	1·77	2·07	1·09
chlorprothixene (3)	6·04	8·22	1·05	1·79	2·42
doxepine (4)	8·84	9·66	2·10	2·39	1·03
dothiepin (5)	8·54	9·05	1·94	2·12	0·99
thiothixene (6)	20·00		5·67		
promazine (7)	16·0		4·52		
chlorpromazine	9·2		2·17		
imipramine	12·45		3·29		
amitriptyline	8·7		2·00		
nortriptyline	27·7		8·55		

1. $K' = \frac{tr\text{-}to}{to}$ 2. $R = \frac{2\,(tt\text{-}tc)}{Wt + Wc}$

to = retention time of unretained solute.
tr = retention time of peak.
tt = retention time of *trans*-component.
tc = retention time of *cis*-component.
Wt = base width of *trans*-peak.
Wc = base width of *cis*-peak.

* Early assignment of the configuration of the isomers was based upon refractive index and dipole moment measurements and was incorrect. Later assignments (Muren & Bloom 1970) were supported by X-ray data (Schaeffer 1967; Post et al 1975).

The assay results for pure and formulated samples of flupenthixol containing a mixture of the *cis*- and *trans*-isomers are recorded in Table 2. Good reproducibility was obtained in the analysis of pure flupenthixol dihydrochloride and a constant ratio of *cis*-*trans* isomers was obtained from replicate determinations. However, the two batches at our disposal showed small but real differences in composition. If formulated into 3 mg tablets a difference in dosage of 1·4 mg to 1·32 mg (~5%) of the *cis*-isomer would result.

In extending this assay to flupenthixol tablets we have found that much care is required in the extraction to ensure significant and reproducible results. Table 2 indicates that two factors must be considered: namely, the efficiency of the extraction in terms of total flupenthixol and the efficiency of the extraction of the respective isomers. Our results

Table 2. H.p.l.c. assay of flupenthixol.

Sample	% *cis*	% *trans*	% recovery*
Batch 1	46·7 (±0·14)	53·3 (±0·14)	
Batch 2	44·0 (±0·12)	56·0 (±0·12)	
1 × 3 mg tablet			
H_2O (5 ml)	51·6	48·4	88·5
H_2O (10 ml)	51·3	48·7	91·7
0·1 M HCl (10 ml)	51·3	48·7	63·7
0·5 M HCl (10 ml)	48·3	51·7	99·3
1 M HCl (5 ml)	49·5	50·5	85·2
1 M HCl	48·9 (±0·15)	51·1 (±0·15)	99·7
2 × 0·5 mg tablet			
H_2O (5 ml)	51·3	48·7	58·5
H_2O (10 ml)	53·1	46·9	64·0
1 M HCl (5 ml)	47·0	53·0	82·0
1 M HCl (10 ml)	46·7	53·3	90·0
1 × 0·5 mg tablet			
H_2O (10 ml)	51·5	48·5	71·4
1 M HCl (10 ml)‡	47·9 (±0·24)	52·1 (±0·24)	100·2

* Based upon nominal weight of flupenthixol in tablets.
† Equivalent to 1·47 (±0·005) mg *cis* and 1·53 (±0·005) mg *trans* per tablet.
‡ Equivalent to 0·240 (±0·001) mg *cis* and 0·261 (±0·001) mg *trans* per tablet.

show that no useful conclusions may be made concerning the *cis*:*trans* ratio unless complete extraction has been achieved. Extraction of tablets (3 mg) using water allows approximately 90% extraction and an apparent *cis* content in excess of 51%. The use of 0·5 or 1 M HCl increases the extraction efficiency and 1 M HCl (10 ml) results in complete extraction. At this point the true *cis* content may be measured and was found to be 48·8%. The extraction of 0·5 mg tablets was even more difficult to control, because of the higher ratio of excipients to drug in this formulation. The use of two tablets to increase the amount of flupenthixol in the extract resulted in low recoveries. One tablet, however, on extraction with 10 ml 1 M HCl, yielded complete extraction and assay of this solution (about 25 μg ml^{-1} in each isomer) indicated the *cis* isomer to account for 47·9% of the flupenthixol available. This represents a small variation between the two batches of tablets examined and is probably not of therapeutic importance.

Another possible explanation to account for the variation in isomer content observed is that the composition of the mixture is changed during analysis, perhaps through acid-catalysed isomerization. However, tests on synthetic mixtures have shown that no change is apparent during the course of the experiment.

These results indicate that an artificially high *cis* value is obtained at low extraction efficiencies and also if water rather than acid is used for the extraction. This suggests that appreciable adsorption of the drug onto the tablet excipients is a real problem in this experiment and that the adsorption characteristics of the *cis*- and *trans*-components are not identical. To evaluate this possibility, the adsorption isotherms of flupenthixol dihydrochloride on activated charcoal were determined. The concentrations were kept below the cmc (Enever et al 1976) to parallel the tablet extraction data. The adsorption of the isomers and the mixture was found to be modelled by the Langmuir isotherm:

$$(c/x) = (1/a) + (bc/a)$$

where c = equilibrium concentration,
x = amount adsorbed g^{-1} of adsorbent,
a, b are constants.

but no difference was observed between the systems ($a = 3{\cdot}11 \times 10^{-3}$ g litre^{-1}, $b = 1{\cdot}39$ litre mol^{-1}). To confirm that no small differences in adsorption were being overlooked, samples were separately checked by h.p.l.c. When aqueous solutions were assayed, the residual solution was found to contain 46·5% of the *cis*-isomer. However, when the corresponding solutions in 1 M HCl were determined the proportion of *cis*- in the supernatant liquid had risen to 48·1%. Although these results cannot be directly related to the tablet assays, they demonstrate clearly that under certain conditions differential adsorption of *cis*- and *trans*-isomers may be apparent.

Whether these results are of therapeutic significance cannot be assessed at present but the methods described here now make available procedures to enable both pharmacological and pharmaceutical properties of the respective isomers to be monitored in detail.

Acknowledgement

We are grateful to Dr J. M. Simister, Mr W. P. J. Evans and Lundbeck Limited for the generous supply of flupenthixol dihydrochloride.

REFERENCES

Bloom, B. M., Tretter, J. R. (1964) Belg. Patent 641498, June 18th, (1966) Chem. Abs. 64: 719c

Bouillin, D. J., Grimes, R. P. J., Orr, M. W. (1975) Br. J. Pharmacol. 55: 555–557

Burt, D. R., Creese, I., Snyder, S. H. (1976) Mol. Pharmacol. 12: 800–812

Crow, T. J., Deakin, J. W. F., Johnstone, F. L. (1977) Nature (London) 267: 18

De Leenheer, A. (1973) J. Chromatogr. 77: 339–350

Dell, H-D., Fiedler, J., Teichgräber, R. (1970) Z. Anal. Chem. 249: 41–44

Dell, H-D., Fiedler, J., Kamp, R. (1971) Ibid. 253: 357–360

Enever, R. P., Li Wan Po, A., Shotton, E. (1976) J. Pharm. Pharmacol. 28: 32P

Enna, S. J., Bennett, J. P., Burt, D. R., Creese, I., Snyder, S. H. (1976) Nature (London) 263: 338–341

Enna, S. J., Bennett, J. P., Burt, D. R., Creese, I., U'Prichard, D. C., Greenberg, D. A., Snyder, S. H. (1977) Ibid. 267: 184

Hobbs, D. C. (1969) Biochem. Pharmacol. 18: 1941–1954

Horn, A. S., Post, M. L., Kennard, O. (1975) J. Pharm. Pharmacol. 27: 553–563

Jørgensen, A., Fredericson Overø, K., Hansen, V. (1971) Acta Pharmacol. Toxicol. 29: 339–358

Jørgensen, A., Gottfries, C. G. (1972) Psychopharmacologia 27: 1–10

Jørgensen, A., Hansen, V., Dahl Larsen, U., Khan, A. R. (1969) Acta Pharmacol. Toxicol. 27: 301–313

Martindale, The Extra Pharmacopoeia (1977a) p 1528 (1977b). p 1213 Pharmaceutical Press

Merck Index (1976). Merck, Sharp and Dohme, Ltd., p. 456.

Miller, R. J., Horn, A. S., Iversen, L. L. (1974) Mol. Pharmacol. 10: 759–766

Meltzer, H. Y., Paul, S. M., Fang, V. S. (1977) Psychopharmacology 51: 181–183

Møller Nielsen, I., Pedersen, V., Nymark, M., Franck, K. F., Boeck, V., Fjallard, B., Christensen, A. V. (1973) Acta Pharmacol. Toxicol. 33: 353–362

Muren, J. F., Bloom, B. M. (1970) J. Med. Chem. 13: 17–23

Petersen, P. V., Møller Nielsen, I. in: Gorden, M., (ed.) 'Psychopharmacological Agents (I),' Academic Press (1964) Pp. 301–324

Post, M. L., Kennard, O., Sheldrick, G. M., Horn, A. S. (1975) Acta Crystallogr. B31: 2366–2368

Post, M. L. Kennard, O., Horn, A. S. (1975). Ibid, B31, 2724–2726

Raaflaub, J. (1967) Arzneim.-Forsch. 17: 1393–1395

Schaeffer, J. P. (1967) Chem. Communs. 743–744

Seeman, P., Lee, T. (1975) Science 188: 1217–1219

Uda, Y., Mizuta, E., Yashiki, T. (1970) J. Takeda Res. Lab. 29: 701–715

Vandemark, F. L., Adams, R. F., Schmidt, G. J. (1978) Clin. Chem. N.Y. 24: 87–91

Weissman, A. (1974) Adv. Biochem. Psychopharmacol. 9: 471–534

Wong, C. K., Cohen, D. M., Munnelly, K. P. (1976) J. Pharm. Sci. 65: 1090–1092