THE DETERMINATION OF THE RELATIVE CONFIGURATION OF MORPHINE, LEVORPHANOL AND *LAEVO*-PHENAZOCINE BY STEREOSELECTIVE ADSORBENTS

By A. H. Beckett and Patricia Anderson

*From Chelsea School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.*3

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The preparation of levorphanol, dextrorphan and morphine stereoselective adsorbents is described. These adsorbents are used to show that the *laevo* isomers of morphine, phenazocine and a related compound, and racemorphan and related morphinans have similar configurations; the analgesic activity of the various compounds resides substantially in these *laevo* isomers.

It has been shown previously that in analgesics of the methadone-type possessing one asymmetric carbon atom, the more active analgesic of each enantiomorphic pair possessed the same configuration[1–3]. This and related information has led to the delineation of analgesic receptor sites of demanding steric requirements[3,4]; the known analgesics have been shown to possess configurations and conformations suitable for association with these sites[3–5].

The analgesic activity of morphine, the morphinans and the benzomorphans has been shown to reside chiefly in the *laevo* isomers of each enantiomorphic pair (see Table I). These structures possess a surface which is complementary to the previously delineated receptor sites; the more analgesically active enantiomorphs would therefore be expected to have the same configurations if the analgesic receptor hypothesis is valid.

The relative configuration of the isomers of the three classes of compounds of Table I has not hitherto been established. Unequivocal chemical methods of synthesis or degradation to produce the evidence for these stereochemical relationships are not currently available. The application of stereoselective adsorbents[10] to the establishing of the stereochemical relationships was therefore investigated.

Experimental

Materials

Sodium silicate solution. Commercial grade material was used—I.C.I. Q.79 containing 8·8 per cent by weight Na_2O; 29·0 per cent by weight SiO_2. Mol. Ratio = 3·4. Sp.gr. at 20° = 1·395.

Levorphanol tartrate. (−)-3-Hydroxy-*N*-methylmorphinan tartrate. $[\alpha]_D^{20} = -14{\cdot}6°$ (C = 3, H_2O) (lit.[12] = −13·8°). Log ϵ = 3·3 at λmax 279 mμ in 5 per cent acetic acid.

Dextrorphan tartrate. (+)-3-Hydroxy-*N*-methylmorphinan tartrate. Log ϵ = 3·3 at λmax 279 mμ in 5 per cent acetic acid.

Benzomorphans. (−)-2′-*Hydroxy*-2,5,9-*trimethyl*-6,7-*benzomorphan.* M.p. 183·5° (lit.[9] 183–184·5°). Log ϵ = 3·29 at λmax 279 mμ in 5 per cent acetic acid.

(+)-2′-*Hydroxy*-2,5,9-*trimethyl*-6,7-*benzomorphan.* M.p. 183° (lit.[9] 183–184·5°). Log ϵ = 3·29 at λmax 279 mμ in 5 per cent acetic acid.

Morphine hydrochloride. M.p. 200° (decomp.) (lit.[13] 200° decomp.). Log ϵ = 3·18 at λmax 285 mμ in 5 per cent acetic acid.

Absorption Measurements

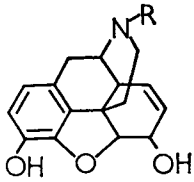
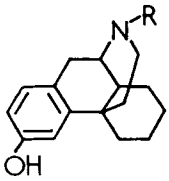
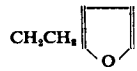
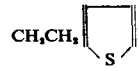
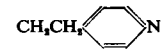
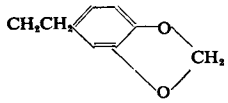
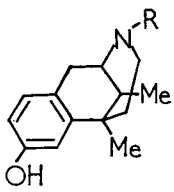
These were made using matched 1 cm. fused silica cuvettes and a Unicam S.P.500 spectrophotometer.

Centrifuge. A "Bara" Gyro Centrifuge was used at 8,500 *g*.

TABLE I

ANALGESIC ACTIVITIES OF ENANTIOMORPHIC PAIRS OF COMPOUNDS OF MORPHINE, MORPHINANS AND BENZOMORPHANS

The recorded analgesic activities are suitable for comparison since they are all obtained by hot plate methods using mice after subcutaneous injection.

Analgesic structure	R	Salt	Isomer	Analgesic activity ED50 mg./kg.	Reference
 (I) Morphine	M	Sulphate	−	10·5	6
			+	almost inactive	7
 (II) Morphinans	Me	Tartrate	−	0·3	8
		HBr	+	44·3	
	CH_2CH_2Ph	HBr	−	0·113	8
		HBr	+	> 100	
		HCl	−	0·010	8
		HCl	+	> 100	
		HCl	−	0·019	8
		HCl	+	> 100	
		Base	−	0·018	8
		Base	+	> 100	
		HCl	−	0·065	8
		HCl	+	> 100	
 (III) Benzomorphans	Me	Base	−	1·7	9
		Base	+	> 20	
	CH_2CH_2Ph	HBr	−	0·11	9
		HBr	+	6·7	

METHODS

Preparation of Adsorbents

Sodium silicate (42 g.) was made up to 200 ml. with distilled water in which 0·5 g. of the reference compound had been dissolved—vigorous stirring being maintained throughout the addition. Approximately 5·7N HCl (130 ml.) was immediately added with continued vigorous stirring. The resultant solution set to a firm gel in 24 hours and the gel was allowed to stand at room temperature for a further 6 days. It was then broken up, spread on sheets of filter paper and allowed to dry in a fume cupboard for 7 days (at this stage the gel was yellow in colour). The gel was subjected to Soxhlet extraction with methanol until colourless, and was then allowed to dry overnight.

The gel was ground in a glass mortar and sieved—those particles between 60 and 200 mesh being retained. These particles were re-extracted with methanol until such time as the reference compound was shown to have been "completely" extracted. The gel was then allowed to dry overnight, sieved to remove any particles of less than 200 mesh which had been formed during the second extraction period and was then ready for use.

A control adsorbent was prepared in exactly the same way but omitting the reference compound. Adsorbents were prepared in this way in the presence of morphine hydrochloride, levorphanol tartrate and dextrorphan tartrate.

Notes on Adsorbent Preparation

1. *Stirring.* Vigorous stirring must be maintained throughout the addition of the acid since inefficient stirring, or slow addition of the acid, was found to cause premature precipitation which produced clumping of the gel.

2. *Drying.* During drying, the filter paper was supported on polythene sheets stretched on wooden frames and supported on a wooden rack. (The acid fumes evolved during drying caused such corrosion of metal frames as to expose the gel to possible contamination). To facilitate drying with the minimum of dust contamination, the frame was placed in a fume cupboard, the air inlet of which was covered by a sheet of muslin.

3. *Preliminary extraction.* At the end of the drying period, the gel was extremely acidic and covered with minute crystals of sodium chloride which gave it a "floury" appearance. The preliminary extraction (*a*) removed the sodium chloride crystals from the surface of the gel, (*b*) reduced the high acidity of the gel (otherwise the gel attacked the metal sieves which were used) and (*c*) left the gel in a more brittle condition, thus facilitating grinding.

4. *Sieving.* To obtain the maximum yield of suitable adsorbent, it was found better to perform numerous sieving and grinding operations rather than reducing the gel to a comparatively fine powder in one operation.

5. *Testing for "complete" extraction.* Complete extraction of the reference compound is never achieved in the true sense as some reference material remains trapped inside the particles of adsorbent; the term is used here to indicate that all the material accessible to the methanol had been removed. In testing for "complete" extraction, methanol (approximately 10 ml.) was removed from the Soxhlet containing the stereoselective adsorbent, evaporated to dryness and the residue, if any, taken up in 5 per cent acetic acid (10 ml.). The resultant solution was centrifuged for 10 minutes and, using in the reference cell a solution prepared

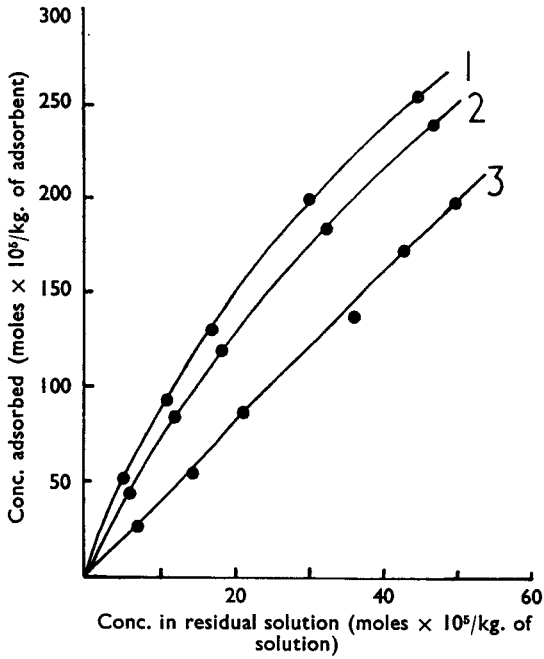


FIG. 1. Adsorption of levorphanol and dextrorphan on levorphanol-selective and control adsorbents. (1) Levorphanol and (2) dextrorphan on levorphanol-selective adsorbent. (3) Levorphanol and dextrorphan on control adsorbent.

similarly from the methanol in contact with the corresponding control adsorbent, spectrophotometric readings were taken in the region of the light absorption peak of the reference compound. Solutions prepared in distilled water were tested for absence of chloride ion.

6. *Ageing of adsorbents.* In general, adsorption measurements were carried out within 2 or 3 weeks of the preparation of the adsorbents.

Measurement of Adsorption

A sample (exactly 1 g.) of the adsorbent (either stereoselective or control), was weighed into a tared glass tube (2·5 × 10 cm.) fitted with

a ground glass stopper. The adsorbent was then washed by rapid shaking with 3 × 10 ml. of a solution of 5 per cent acetic acid, over a period of 1 hour. After shaking, the adsorbent was allowed to settle, and as much as possible of the acid removed without disturbing the adsorbent. This was achieved by using a fine suction tube, the end of which was bent so as to be parallel to the surface of the liquid.

After this preliminary washing, a further 10 ml. of acetic acid solution was added and the adsorbent shaken for a further 1 hour. A portion of the acid was then removed, centrifuged, and absorption measurements taken around the peaks for the compound which was going to be adsorbed

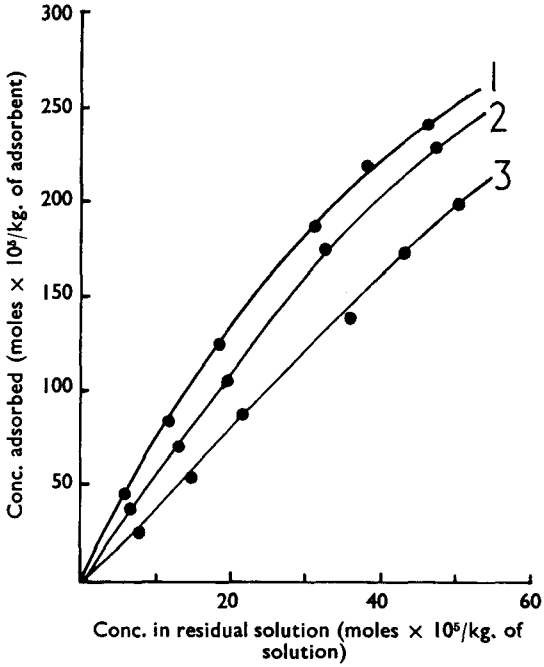


FIG. 2. Adsorption of dextrorphan and levorphanol on dextrorphan-selective adsorbent and control adsorbent. (1) Dextrorphan and (2) levorphanol on dextrorphan-selective adsorbent. (3) Dextrorphan and levorphanol on control adsorbent.

and for the reference compound. In general, zero readings were obtained, but if not, the washing procedure was continued until zero readings were obtained.

After the final washing, as much of the acid solution as possible was removed, and the weight adjusted with 5 per cent acetic acid to that amount which, when the compound to be adsorbed (in 5 per cent acetic acid) was added, it gave the required starting concentration, and a solution to adsorbent ratio of 10 : 1 by weight. The adsorbent and solution were then shaken for 1 hour, by which time equilibrium was reached in these systems.

After 1 hour, the adsorbent was allowed to settle, the supernatant liquid decanted off and centrifuged for 10 minutes. The centrifugate was pipetted off and its concentration determined spectrophotometrically.

By the above method, duplicate readings for a particular adsorbent in the same experiment using separate tubes and the same starting concentration of adsorbate, showed good agreement, e.g. 94 of 109 pairs of readings agreed to within 3 per cent for the amount of material adsorbed.

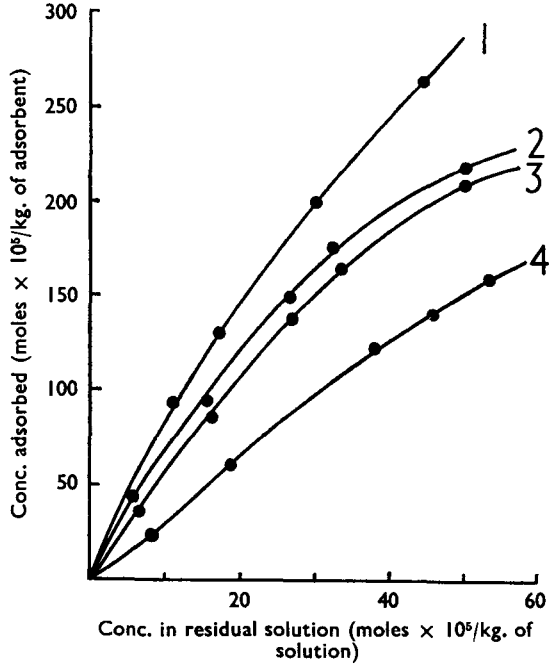


FIG. 3. Adsorption of levorphanol and (+) and (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan on levorphanol selective and control adsorbents. (1) Levorphanol, (2) (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan, and (3) (+)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan, on levorphanol-selective adsorbent. (4) (+) and (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan on control adsorbent.

RESULTS AND DISCUSSION

A silica gel prepared in the presence of levorphanol (II; R = Me) gave, under the prescribed treatment, an adsorbent which adsorbed levorphanol better than its enantiomorph, dextrorphan, and adsorbed both isomers better than did an adsorbent obtained from a gel prepared in the absence of levorphanol (see Fig. 1). Batch to batch variation occurred in the adsorptive power of the adsorbents but the relative adsorption pattern of the isomers was always similar. A gel prepared in the presence of dextrorphan gave an adsorbent which adsorbed dextrorphan better than it did levorphanol (see Fig. 2). The adsorptive power of the adsorbents varied with the time, temperature and humidity

of the storage conditions but the relative adsorption pattern for the isomers on a particular adsorbent did not.

It is considered that the above preparation of stereoselective adsorbents results in "footprints," at the surface of the adsorbent particles, of the reference molecules which were present during the gel formation. The extraction procedure is considered to strip the organic molecules from

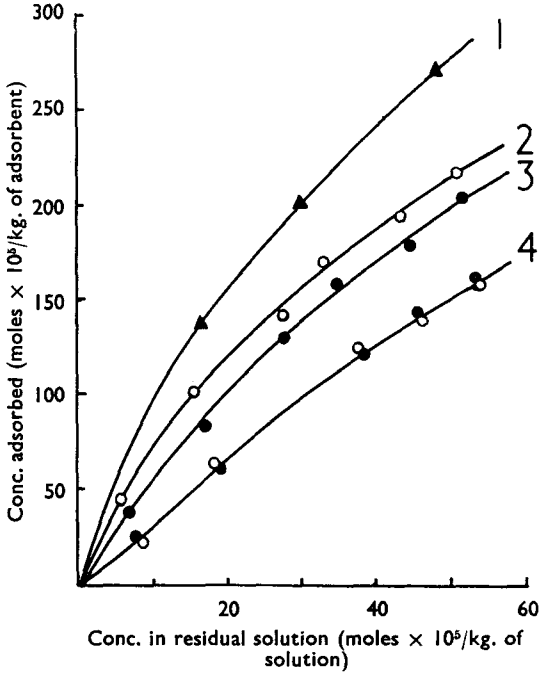


FIG. 4. Adsorption of dextrorphan and (+) and (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan on dextrorphan-selective and control adsorbents. (1) Dextrorphan, (2) (+)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan, and (3) (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan on dextrorphan-selective adsorbent. (4) (+) and (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan on control adsorbent; ○ represents the (+)- and ● the (−)-isomer.

the surface layers to leave imprints and partial imprints with configurational integrity in the hydrated silica surface. A less likely explanation[11] for the selectivity of the adsorbent is that the unextractable (under these conditions) organic molecules left embedded inside the adsorbent set up active points for the association of identical and related molecules. (Detailed discussion of these explanations will be presented elsewhere.)

Irrespective of which explanation be correct, we have already shown that adsorbents which exhibit stereoselectivity may be used for configurational assignments because molecules of like configuration are adsorbed more readily than those of unlike configuration. Consequently an isomer not too dissimilar in structure from levorphanol and of like configuration will be adsorbed better on a levorphanol selective adsorbent

than will its stereoisomer; the opposite adsorption pattern for the two stereoisomers should obtain using a dextrorphan selective adsorbent. In Figure 3 is presented the adsorption of (−)- and (+)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan (III; R = Me) on a levorphanol selective adsorbent; the (−)-isomer is adsorbed more than the (+)-isomer. Figure 4 shows that (+)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan (III; R = Me) is adsorbed more strongly than its enantiomorph

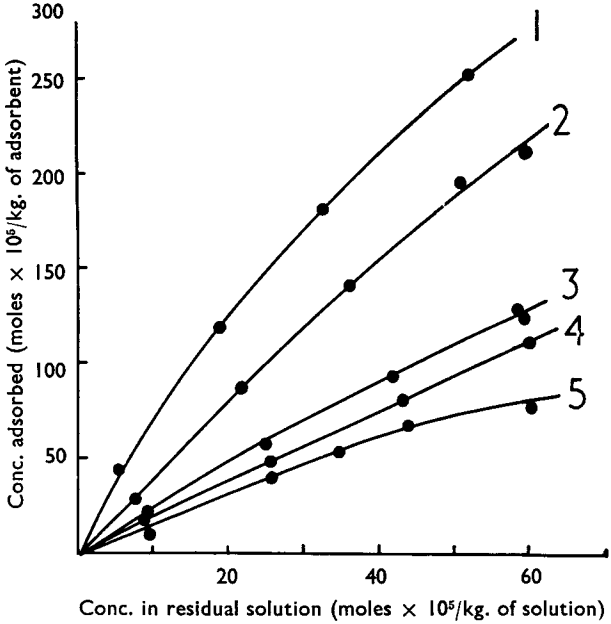


FIG. 5. Adsorption of dextrorphan, levorphanol, and morphine on dextrorphan- and levorphanol-selective adsorbents and on control adsorbent. (1) Dextrorphan on dextrorphan-selective adsorbent and levorphanol on levorphanol-selective adsorbents. (2) Dextrorphan and levorphanol on control adsorbent. (3) Morphine on levorphanol-selective adsorbent. (4) Morphine on dextrorphan-selective adsorbent. (5) Morphine on control adsorbent.

on a dextrorphan selective adsorbent. Similar adsorbents prepared at different times and used after different storage times gave similar relative adsorption patterns for the isomers despite differences in the actual adsorptive power of the adsorbents. It follows that (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan (III; R = Me) and levorphanol (II; R = Me) have similar configurations.

Because (−)-phenazocine (III; R = CH_2CH_2Ph) is prepared from (−)-2′-hydroxy-2,5,9-trimethyl-6,7-benzomorphan (III; R = Me) and (+)-phenazocine from the (+)-*N*-methylbenzomorphan by a route not involving the asymmetric centres, identical configurations for levorphanol and (−)-phenazocine are established.

Adsorbents obtained from gels prepared in the presence of (−)-morphine did not exhibit pronounced adsorptive power compared with

that of control adsorbents. Such weak morphine selective adsorbents adsorbed levorphanol slightly more strongly than dextrorphan. The configurational similarity of (−)-morphine (I; R = Me) and levorphanol was thus indicated but further evidence was required. In Figure 5 the uptake of (−)-morphine on levorphanol and dextrorphan selective adsorbents of similar adsorptive power is presented; (−)-morphine is adsorbed more strongly on the levorphanol selective adsorbent than on the dextrorphan selective one. Thus levorphanol and (−)-morphine have similar configurations.

The other (−)-morphinan isomers (II) shown in Table I are known to have configurations identical with that of levorphanol because of their method of preparation from the same precursor isomer. Consequently all the (−)-morphinan isomers shown in Table I have configurations similar to that of (−)-morphine.

The analgesic activity of morphine (I), phenazocine (III; R = CH_2CH_2Ph) and its related *N*-methyl analogue (III; R = CH_3), and racemorphan and its related *N*-alkyl analogues (II), resides substantially in the *laevo* isomers which are shown above to have similar configurations. The importance of the configurational requirements of the analgesic receptor site thus receives further support.

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After Miss Anderson presented the paper there was a DISCUSSION. The following points were made.

It was possible by use of stereoselective adsorbents to line up the various compounds which had similar configurations. If such arrangements agreed with pharmacological considerations, support would be provided for the analgesic receptor site hypothesis. The authors did not agree with the alternative explanation by Canadian workers. The technique was being used solely to establish configuration. Italian workers had separated isomers by means of stereoselective adsorbents.