Antinociceptive potency ratios for (+)- and (−)-4-dimethylamino-2,2-diphenylpentanoate (the ethyl ester analogue of methadone)

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Early in the post-war investigations of acyclic analgesics of the 3,3-diphenylpropylamine class the unexpected discovery was made that the more active antipodal form of the ethyl ester analogue of methadone (Ia) was formed from the nitrile precursor (Ic) of the *less* active (+)-isomer of methadone (Ib) (Pohland et al

I. $Me_2NCHMeCH_2CPh_2R$
(a) R = CO_2Et
(b) R = COEt
(c) R = CN
(d) R = CHO
(e) R = CH_2OH

1949). Data gathered over the past ten years are now reported which confirm potency rankings of (+)-, (−)-, and (±)-forms of 'methadone ester', give evidence of the morphine-like pharmacological classification of the dextro isomer, and remove doubts about the configurational relationships of antipodal forms of methadone and its ethyl ester analogue. Original and novel pharmacological data are summarized in Table 1. The isomeric potency ratios are of modest order (3–5:1) but consistently show the (+)-isomer to be the more active antipode with a potency close to that of pethidine. In rats the (+)-ester (20 mg kg^{-1}, i.v.) produced the typical morphine-like syndrome of lead pipe rigidity, reduced reaction to noxious stimuli, and respiratory depression, while in mice the same compound resulted in hot-plate activity, mydriasis, the Straub tail phenomenon and excitation –all effects were antagonized by nalorphine.

Although the conversion of optically active methadone nitriles (Ic) to the (+)- and (−)- ethyl ester analogues (Ia) involves reactions at carbon functions remote from the chiral centre, the possibility of configurational change may not be excluded entirely. In one preparative run, dimethylamine hydrogen sulphate (m.p. 141–142 °C. Found: C, 16·84, H, 6·1. $C_2H_9NO_4S$ requires C, 16·78, H, 6·3%) was isolated indicating that an elimination-addition equilibrium mechanism may operate under the low pH conditions of the reaction (H_2SO_4–H_2O followed by $SOCl_2$–EtOH) which must disturb the asymmetric centre. Proof of retention of configuration was obtained, however, by a correlation sequence that avoided the use of acidic reagents. Reduction of the (−)-nitrile (Ic) with lithium aluminium hydride was carried out in two stages to yield first the (−)-aldehyde (Id) and then the (−)-primary alcohol (Ie). The latter was identical (i.r., m.p. and mixed m.p., optical rotation) with the product obtained by reduction of the (−)-ester (Ia), itself derived from the (−)-nitrile by use of acidic reagents. Details of these reactions are given in Table 2. Correlation experiments involving attempted cleavage of the ethoxycarbonyl substituent by sodamide, or the sequence $RCN \rightarrow RCH_2NH_2 \rightarrow RCH_2OH$ (a racemic pro-

Table 1. Antinociceptive activities of (±), (+) and (−)-ethyl 4-dimethylamino-2,2-diphenylpentanoate in rodents.

Form	ED50[a]	Rat tail burn test[b] Dose	% activity
(±)-HCl	18·0	10·0	20
(+)-HCl	5·4	10·0	20
(−)-HCl	24·0	60·0	3
(±)-Methadone	1·6		
(±)-HCl	6·3[c]		
(+)-HCl	4·2		
(−)-HCl	11·2		
Pethidine	4·7		
(±)-HCl	22·0[d]		
(−)-HCl	40·0		
(+)-HSO_4	19·0		
(−)-HSO_4	40·0		
Pethidine	23·0		

a mg kg^{-1}, s.c., mice, hot-plate (Eddy et al 1956).
b mg kg^{-1}, s.c. activity compared with (±)-methadone at 2·0 mg kg^{-1} (Pohland et al 1949).
c mg kg^{-1}, s.c., CDGP mice, hot-plate (data from National Institute of Health, Bethesda).
d mg kg^{-1}, s.c., mice, hot-plate (data from Janssen Pharmaceutica).

Table 2. Substances isolated during stereochemical correlation experiments.

Id HNO_3 m.p. 186–9 °C (lit. [a]188–9 °C) $[\alpha]_D^{20}$ — 35·9 (c, 1·4 in EtOH) C = O 1705 cm^{-1} (Nujol mull).
Ie base[b] m.p. 116–7° from light-petroleum (60–80 °C) $[\alpha]_D^{20}$ — 54·7 (c, 15·2 in EtOH). Found: C, 80·97; H, 9·0. $C_{19}H_{25}NO$ requires C, 80·56; H, 8·83%.
Ie HCl m.p. 204–5 °C $[\alpha]_D^{20}$ — 62·3 (c, 0·3 in H_2O). Found: C, 71·07; H, 8·11; N, 4·32. $C_{19}H_{26}ClNO$ requires C, 71·56; H, 8·14; N, 4·38%.

a (Perrine & May 1954). 1 mole (−)-Ic treated with 0·3 mole lithium aluminium hydride (1·5 molar solution in ether).
b (−)-Id treated with excess of lithium aluminium hydride.

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duct formed after acid deamination of the amine) were unsuccessful.

If the antipodal potency ratios for the (+)- and (—)-esters (Ia) reflect receptor events (distribution factors have been shown to have only a minor influence on the potencies of the closely related methadone enantiomers) (Sullivan et al 1975), then the low values observed together with the reversal of receptor stereoselectivity relative to that shown towards methadone and many of its relatives (Casy 1973) provide further evidence of the conformational mobility of the methadone class of analgesic, a feature recently stressed by Henkel et al (1974, 1976).

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High sensitivity solid-phase exchange radioimmunoassay for morphine

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In recent studies (Fishman & Fishman 1974; Castaneda & Liao 1975) immobilized antibodies have been used as a probe in determining the quantitative and qualitative characteristics of steroid hormone binders which compete with the antibody for the same ligand. We felt that such methodology could also be applied to the study of the opiate receptor, and to that end we have raised and immobilized a morphine antibody. In the course of testing the immobilized system it was applied to the solid phase radioimmunoassay RIA of morphine and related opiates. A note by Steiner & Spratt (1978) prompts us to report the results obtained by us with our system insofar as RIA of morphine is concerned. In these applications the methodology we report exhibits several distinct advantages.

The first use of radioimmunoassay (RIA) for the assessment of a drug of abuse (morphine) concentration in body fluids was by Spector & Parker in 1970. Since then radioimmunoassays have been developed for methadone (Bartos et al 1977), barbituates (Spector & Flynn 1971), amphetamines (Cheng et al 1973), and other abused drugs (Castro & Malkus 1977). Other techniques which include haemoagglutination inhibition assays (Adler & Liu 1971), spin label assays (Leute et al 1972), and enzyme linked immunoassays (Rubenstein et al 1972) have also been developed for the measurement of morphine and its congeners.

The use of immobilized antibodies in other radioimmunoassays, has previously been reported (Catt et al 1968; Goldstein et al 1972; Castaneda & Liao 1975). One of the main advantages in using this technique is to eliminate the troublesome step of separating the complexed from the uncomplexed ligands since no precipitant or absorbant need be added after the initial binding of ligand.

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Antiserum preparation. Morphine-6-succinylbovine serum albumin (M-6HS-BSA) was prepared by modification of published procedures. Morphine (free base) was heated under reflux with succinic anhydride (Simon et al 1972) to give the morphine 6-hemisuccinate which after purification was conjugated with BSA using the mixed anhydride procedure described by Wainer et al (1972). The antigen which contained 9·2 mol ligand per mol was dissolved in phosphate buffered saline and emulsified with an equal volume of Freund's complete adjuvant to provide a concentration of 0·5 mg ml^{-1}. New Zealand white rabbits were injected with 1·6 ml of this suspension, 0·4 ml being injected into each footpad. Booster injections were administered three to five weeks later, and the rabbits were bled at seven day intervals. Aliquots of the serum obtained from the bleeding were stored and frozen. Hapten binding capacity of the antiserum samples at different dilutions was determined by incubation with [6-^{3}H]morphine (New England Nuclear) at 60 °C for 10 min and then overnight at 4 °C. The charcoal absorption technique (Odell et al 1975) was then used to separate complexed from free ligand. The charcoal was sedimented by centrifugation, and the supernatant was decanted and counted in a Packard liquid scintillation counter.

Immobilized antibody (IAb). Strips of water swollen unsintered polyvinylidene fluoride film (Roche Diagnostics, Nutley, N.J.), 0·6 × 3·0 cm were each submerged in 1·0 ml of antiserum solution (1 : 1000 dilution), and