Our concern related to the validity of the ferrocitrate assay as a stability method for isoproterenol in a nonaqueous aerosol preparation[1] with thonzonium bromide, ascorbic acid, alcohol, and propellants. In order to ascertain whether or not degradation of isoproterenol in this preparation furnished a product with an intact catechol function, we subjected aged samples to thin-layer chromatography and sprayed the developed chromatograms with Doty reagent prepared according to the NF (7). (The use of this reagent as a selective spray for detection of catechol derivatives on chromatograms has not been described previously. A fuller description of its application will be published elsewhere.)

The aerosol containers were chilled by immersion in a freezing bath of solid carbon dioxide–acetone, opened with pliers, then allowed to warm to room temperature to evolve propellants. The remaining solution was diluted with an equal volume of absolute methanol, and 25-μl. volumes of it and a 600 mcg./ml. solution of reference isoproterenol HCl were spotted 3 cm. from the bottom edge of a commercial precoated 20 × 20 cm. thin-layer plate consisting of a 0.25-mm. thickness of microcrystalline cellulose without binder or phosphor on glass.[2] Before use, the plates were developed in the solvent to about 15 cm. from the starting line and air dried to remove contaminants from the plate. Pretreatment of the plate in this manner eliminated an apparent second front across the chromatogram which resulted in artifactual spots such as those described by Choulis (8). The chromatogram was developed to about 15 cm. from the starting line with a mixture of 2-propanol and 0.1 *N* hydrochloric acid (5:1). After air drying and spraying with the Doty reagent, only one purple spot was obtained at R_f 0.4, corresponding to intact isoproterenol. The detection limit for isoproterenol HCl or epinephrine HCl is below 0.3 mcg. (2% of the amount spotted from the formulation). A volume of 0.5 μl. of 0.6 mg./ml. epinephrine HCl (0.3 mcg.) was added to a sample preparation spot. It was readily detected at R_f 0.2 after chromatographic separation and treatment with Doty reagent. Thus, the method was shown to be capable of separating isoproterenol from a closely related catecholamine and detecting at least 2% of the amount spotted.

The procedure was applied to isoproterenol and thonzonium bromide aerosol samples stored at room temperature for more than 5 years. Examination of stability data obtained for five lots of the formulation during this time revealed no consistent pattern of degradation. Instead, it appears that each sample reacts to the extent of the oxygen present and remains substantially unchanged thereafter. Although some degradation was evident in all of the stability samples, isoproterenol content remained satisfactory throughout the testing period.

[1] Marketed as Nebair by Warner-Chilcott Laboratories, Morris Plains, N. J.

[2] Uniplate coated with Avicel, obtained from Analtech, Inc.

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Received December 26, 1967.
Accepted for publication February 16, 1968.

Keyphrases

Isoproterenol, aerosol—stability assay
Doty reaction—validity confirmed
Catechol oxidation detection—Doty reagent
TLC—analysis, separation

Absolute Stereochemistry and Analgesic Potency of Prodine Enantiomers

Sir:

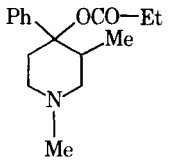
Stereochemical factors are known to be of importance in analgesic action (1). Among the structures in this well-known class of biologically active compounds, α and β-prodine (Ia and Ib, respectively) (2) distinguish themselves by being highly potent synthetic analgesics possessing two asymmetric centers. While the relative stereochemistries of Ia and Ib are known (3), the absolute configurations and potencies of the optically pure enantiomers have not been reported and are of great interest because they possess an asymmetric center in common with isomethadone (4). In this communication evidence is presented which permits assignment of the complete absolute stereochemistry of these enantiomers.

Treatment of racemic IIa (2) with an equivalent of (+)-tartaric acid in methanol–acetone

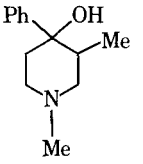
afforded the acid tartrate salt, m.p. 161–162°, $[\alpha]_D^{22}$ + 13.5° (c 1, H_2O), from which the amine, m.p. 89–90°, $[\alpha]^{22}$ + 10.2° (c 1, acetone), was regenerated. This was transformed to the methiodide, m.p. 169–170°, $[\alpha]_D^{22}$ + 1.6° (c 1, EtOH), which was converted to the quaternary hydroxide and then heated at 170° for 1 hr. The structure of the elimination product, III, $[\alpha]_D^{26}$ + 59.8° (c 2, Et_2O), (75% yield) was confirmed by the NMR spectrum which showed a doublet methyl resonance at 0.82 p.p.m. (J = 6.5 c.p.s.) and three olefinic proton resonances; H_a = 5.13 p.p.m., H_b = 5.44 p.p.m. (J_{ab} = 2 c.p.s.), H_c = 6.40 p.p.m. (J_{bc} = 17 c.p.s., J_{ac} = 10 c.p.s.). Hydroxylation of III with osmium tetroxide followed by *in situ* cleavage of the triol with sodium metaperiodate afforded (−)-IV which was isolated as the maleate salt, m.p. 127–129°, $[\alpha]_D^{20}$ − 52.6° (c 1, EtOH). The physical properties were found to be in accord with those of the maleate salt, m.p. 127–128°, $[\alpha]_D^{20}$ − 53.2° (c 1, EtOH), derived from authentic IV (5) which has been determined to be in the (*R*)-series. Esterification of (+)-IIa with propionyl chloride afforded (+)-α-prodine hydrochloride, m.p. 191–193°, $[\alpha]_D^{21}$ + 26.5° (c 1, EtOH). Employing the same procedure, (−)-α-prodine hydrochloride, m.p. 192–193°, $[\alpha]_D^{21}$ − 27.9° (c 1, EtOH), was prepared from (−)-IIa, m.p. 88–89°, $[\alpha]_D^{21}$ − 10.7° (c 1, acetone), obtained by using (−)-tartaric acid in the resolution of the racemic alcohol. The IR spectra of both antipodes were superimposable and virtually identical to that of the racemate.

The above chemical correlation establishes (+)-Ia·HCl as having the (3*R*) configuration. Since it is known (3) that the phenyl and methyl groups in Ia have a *trans* relationship, it follows therefore that the complete stereochemistry of (+)-α-prodine hydrochloride is (3*R*:4*S*) and is depicted by structure VIa. Due to its enantiomeric relationship with (+)-Ia·HCl, the stereochemistry of (−)-α-prodine hydrochloride is designated as (3*S*:4*R*).

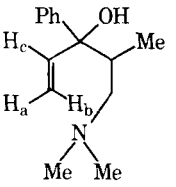
Treatment of (±)-IIb (2) with one equivalent of (−)-dibenzoyltartaric acid in H_2O–MeOH afforded the acid dibenzoyltartrate salt, m.p. 163–164°, $[\alpha]_D^{22}$ − 42.6° (c 1, MeOH), from which (+)-IIb, m.p. 136–137°, $[\alpha]_D^{20}$ + 74.5° (c 1, acetone) was obtained. Employing (+)-dibenzoyltartaric acid in an identical procedure gave (−)-IIb, m.p. 136–137°, $[\alpha]_D^{20}$ − 75.0° (c 1, acetone). Dehydration of (+)-IIb with aqueous HCl yielded (−)-V, $[\alpha]_D^{22}$ − 115° (c 1, Et_2O), whose IR and NMR spectra were identical to that of the racemic olefin (6). Pyrolysis of (+)-Ia according to the procedure of Diamond (7)



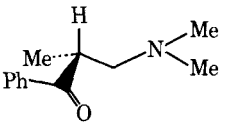
Ia *trans*-Ph:Me
Ib *cis*-Ph:Me



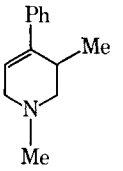
IIa *trans*-Ph:Me
IIb *cis*-Ph:Me



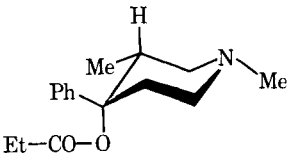
III



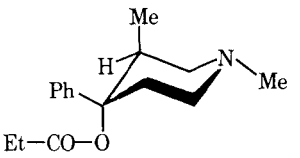
IV



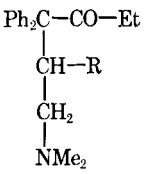
V



VIa



VIb



VII, R = Me
VIII, R = H

afforded (+)-V, $[\alpha]_D^{22}$ + 93.2° (c 1, Et_2O), which possessed IR and NMR spectra identical to that of enantiomeric and racemic material. Esterification of (+)-IIb with propionyl chloride gave (+)-Ib·HCl, m.p. 186–188°, $[\alpha]_D^{20}$ + 74.5° (c 1, EtOH). Employing (−)-IIb in an identical procedure afforded (−)-Ib·HCl, m.p. 186–188°, $[\alpha]_D^{20}$ − 73.7° (c 1, EtOH). The IR spectra of both enantiomers were virtually identical to that of the racemate.

The preceding stereochemical correlation and the known *cis* relationship (3) between phenyl and methyl groups in β-prodine permits the configurational assignment of (+)- and (−)-Ib·HCl as (3*S*:4*S*) (VIb) and (3*R*:4*R*), respectively.

It was found that most of the analgesic activity of both α- and β-prodine resides in the (+)-antipodes (Table I). The analgesic potency of the diastereomeric tartrate salts of Ib has been reported (8). However, in view of the reported low enantiomeric potency ratio and our inability to effect complete optical resolution by this

TABLE I—ANALGESIC POTENCY OF PRODINE ENANTIOMERS

Compound	ED_{50}[a], mg./Kg.
dl-α-Prodine HCl	1.7
(+)-α-Prodine HCl	0.90
(−)-α-Prodine HCl	22.0
dl-β-Prodine HCl	0.35
(+)-β-Prodine HCl	0.25
(−)-β-Prodine HCl	2.6
3-Desmethylprodine HCl	1.3
Meperidine HCl	12.0

[a] Analgesic activity was determined by a modified hot plate method (13).

method, it is very probable that Ib was only partially resolved.

It is significant that the C-3 asymmetric centers in the more potent isomers have opposite configurations. This, coupled with the observation that desmethylprodine (9) possesses greater potency than either of the less active prodine isomers (Table I) suggests that the 3-methyl group does not contribute to the drug-receptor interaction in a totally positive fashion. The difference in potency between (+)-Ia and (+)-Ib most probably is related to different brain levels[1] and it is conceivable that the lower potency of desmethylprodine, when compared to the more active antipodes, may largely be due to lower brain levels rather than to the absence of involvement of the 3-methyl group at the receptor level.

It is noteworthy that the more active enantiomers of isomethadone (VII) (10) and Ia are configurationally related (11). Since the desmethyl analog (VIII) (12) possesses potency which is comparable to VII, there exists the possibility that in this case the methyl group of (*S*)-isomethadone is also playing primarily a passive role in the interaction with analgesic receptors.

[1] Preliminary experiments indicate that the brain levels of β-prodine in rats are greater than those of the α-isomer and that this is the primary factor determining the potency difference between these diastereomers.

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Received November 15, 1967
Accepted for publication February 9, 1968.

This investigation was supported by research grant NB 05192 from the National Institutes of Health.

The authors gratefully acknowledge the generosity of Dr. W. E. Scott, Hoffmann-LaRoche, Inc., for providing a supply of α- and β-prodine alcohol, and Dr. A. Pohland, Lilly Research Laboratories, for the specimen of (−)-β-dimethylamino-α-methylpropiophenone hydrochloride. We also are indebted to Dr. H. Kupferberg of the University of Minnesota for his aid in the pharmacological testing.

Keyphrases

Prodine enantiomers—absolute sterochemistry
Optical rotation—structure
IR spectrophotometry—structure
NMR spectroscopy—structure
ED_{50}—prodine enantiomers
Absolute configuration—analgesic activity

4,4′-Dihydroxybibenzyl, a Reduction Metabolite of *trans*-Stilbene

Sir:

Our investigations of the metabolism of certain stilbenes has revealed a unique conversion of *trans*-stilbene in rabbits (2.1%)[1] to 4,4′-dihydroxybibenzyl.

The above metabolite (originally designated X) was found in a phenolic fraction obtained from an ether extract of urine of rabbits, which had been administered *trans*-stilbene (25 mg./Kg., i.m.). Paper, thin-layer, and vapor phase chromatography (VPC) (1) of this phenolic fraction disclosed the presence of the *trans*-stilbenes, 4-hydroxy-, 4,4′-dihydroxy-, 3-hydroxy-4-methoxy-, and 4-hydroxy-3-methoxystilbene, and X, which was not comparable to these or other simple phenolic stilbenes. A 2-mg. sample of the trimethylsilyl (TMS) either of X, obtained by preparative VPC, was subjected to IR, UV, and mass spectral analysis.

The IR spectrum of the TMS derivative of X,

[1] Determined by VPC (TMS ether). Chromatographic analysis of urine obtained from guinea pigs administered *trans*-stilbene has indicated an even greater (10.6%) conversion to 4,4′-dihydroxybibenzyl.