mainder of the procedure was essentially the same as that used in the preparation of IIb; yield of hygroscopic IIa, 28 g. (73%), m.p. 132–134°. Rearrangement of 28 g. of IIa (dried at 60° *in vacuo*) was effected as described for IIb and gave 23 g. of impure IIIa. This and 200 ml. of 48% hydrobromic were kept at a bath temperature of 140–150° for 24 hr. and the resultant Va isolated as described for Vb; yield 9.8 g. (43%) of crude Va; 6.8 g. m.p. 214–217°, after a recrystallization from acetone or methanol, λ_{max}^{Nujol} 6.14(w), 6.30(s) μ.

Anal. Calcd. for $C_{18}H_{24}ClNO$: C, 68.19; H, 8.58. Found: C, 68.42; H, 8.60.

α-5,9-Dipropyl-2'-methoxy-2-methyl-6,7-benzomorphan Methiodide.—Methanol (45 ml.), 5 g. of Vb and 80 ml. of 3% ethereal diazomethane were stirred to solution (4–6 hr.). An additional 80 ml. of diazomethane solution was added and the mixture kept at 25° for 2–3 days. Solvents were distilled finally *in vacuo* and the residue evaporatively distilled at 0.2 mm. (bath temperature 145°). The 4.3 g. of distillate and methyl iodide in acetone gave the methiodide; prisms, m.p. 245–247°.

Anal. Calcd. for $C_{21}H_{34}INO$: C, 56.88; H, 7.95. Found: C, 57.06; H, 7.85.

1,2-Dipropyl-7-methoxy-1-(2-dimethylaminoethyl)-1,2,3,4-tetrahydronaphthalene (VIb) Hydrochloride.—The above methiodide (4.3 g.), 4.3 g. of sodium hydroxide and 43 ml. of water were refluxed for 6 hr., cooled and extracted with ether. Evaporation of the ether left 2.5 g. of oil which, with 0.2 g. of platinum oxide and 30 ml. of methanol, absorbed 1 molar equivalent of hydrogen in 30 min. The filtered solution was evaporated to dryness and the residue converted to the hydrochloride (acetone–ether–dry hydrogen chloride).

Anal. Calcd. for $C_{21}H_{38}ClNO$: C, 71.3; H, 10.3. Found: C, 71.5; H, 10.4.

1,2-Dipropyl-7-methoxynaphthalene Picrate.—One gram of VIb and 1.0 g. of 5% palladium–charcoal were mixed intimately in a vented test tube which was then immersed in an oil bath, preheated to 250°. The temperature of the bath was raised to 315° during 10 min. and kept at this temperature ±5° for another 20 min. The cooled mixture was extracted 3 times with ether and these extracts were washed with dilute hydrochloric acid. Drying and evaporation of the ether and evaporative distillation at 100–110° (0.2 mm.) gave 0.6 g. of hydrocarbon which with 0.6 g. of picric acid and 3–5 ml. of ethanol (warming to solution) yielded, after cooling to −15°, the crystalline picrate; orange needles from methanol, melting at 68–69° to a melt which did not flow freely until 100°.

Anal. Calcd. for $C_{23}H_{25}N_3O_8$: C, 58.58; H, 5.35. Found: C, 58.45; H, 5.33.

Ultraviolet maxima (in ethanol) of the crude distillate above or hydrocarbon prepared from the pure picrate were at 234, 279, 288, 315 and 330 mμ. These and extinction coefficients were consistent with the 7-methoxy-1,2-dialkylnaphthalene structure.[6]

7-Methoxy-1-(2-dimethylaminoethyl)-1-propyl-1,2,3,4-tetrahydronaphthalene (VIa) Hydrochloride.—This compound was prepared essentially as described for VIb. The intermediate **2'-methoxy-2-methyl-5-propyl-6,7-benzomorphan methiodide** crystallized from acetone–methanol; m.p. 199–204°. It was dried at 100° for analysis.

Anal. Calcd. for $C_{18}H_{28}INO$: C, 53.89; H, 7.03. Found: C, 54.06; H, 7.32.

The VIa hydrochloride prepared from this methiodide crystallized from acetone in needles, m.p. 202–204°; yield from Va 70%. It was dried at 100° for analysis.

Anal. Calcd. for $C_{18}H_{30}ClNO$: C, 69.33; H, 9.70. Found: C, 69.36; H, 9.76.

Stereochemistry of the Interaction of Enantiometic 1,3-Dioxolane Analogs of Muscarone with Cholinergic Receptors[1]

B. BELLEAU AND J. PURANEN

Department of Chemistry, University of Ottawa, Ottawa, Ontario

Received November 19, 1962

Starting from D-isopropylidene glycerol, the synthesis of L(+)-*cis*-2-methyl-4-trimethylammoniummethyl-1,3-dioxolane iodide (XVI) and its enantiomer (VIII) is described as outlined in Charts 1 and 2. The relative configurations were established by direct comparison of the key intermediates VI and XIV with corresponding racemates of previously established configurations. Comparison of the cholinomimetic activity of VIII with that of XVI revealed the latter to be approximately 100 times more active than the former and 6 times more potent than acetylcholine. It is pointed out that these observations are not consistent with Waser's interpretation of the active conformation of D-muscarone. The inversion of the optical specificity of the receptors toward the enantiomers of muscarone but not toward the dioxolane analogs VIII and XVI is accounted for if the presence of an accessory nucleophilic site on the receptor is postulated.

In the first paper of this series,[1] the synthesis, stereochemistry and cholinomimetic activity of quaternary salts of the 1,3-dioxolane series (the Fourneau series[2]) was reported. The effect of optical isomerism on activity in this group was also studied in a preliminary fashion[1] and the results suggested that the enantiomers of *cis*-2-methyl-4-trimethylammoniummethyl-1,3-dioxolane iodide (*cis*-F2268) (I, R = CH_3) should be of special interest because optimum activity is associated with the *cis* configuration. Resolution experiments having produced negative results, the synthesis of the desired enantiomers (VIII) and (XVI) was approached using a starting material of known absolute configuration. We have shown[1] that the 1,3-dioxolane I (R = CH_3) referred to as F2268 in the literature[2] consists of a 60:40 mixture of *cis*- and *trans*-isomers. The synthesis of pure *dl-cis*- and *dl-trans*-F2268 was successfully accomplished and the configurations rigorously established.[1] We now wish to report the synthesis and cholinomimetic activity of the enantiomers of *dl-cis* F2268.

Starting from D-isopropylidene glycerol, the sequence described in Chart I was applied to the synthesis of optically pure D(-)(VIII) (D-*cis*-F2268). Intermediates (III) and (IV) were described in part I.[1] We had observed[1] previously that the separation of *cis*, *trans* isomers in the 1,3-dioxolane series could be accomplished best when a trichloromethyl substituent rather than a methyl group was present at position 2. As expected, the reaction of chloral with IV led to a 60-40 mixture of D-*trans*-(V) and D-*cis*-(VI) from which pure VI could be separated by crystallization albeit

(1) Published as part II of the series entitled "Studies on the Chemical Basis for Cholinomimetic and Cholinolytic Activity." For part I, see D. Triggle and B. Belleau, *Can. J. Chem.*, **40**, 1201 (1962).

(2) J. P. Fourneau, D. Bovet, F. Bovet, and G. Montézin, *Bull. Soc. Chim. Biol.*, **26**, 134, 516 (1944).

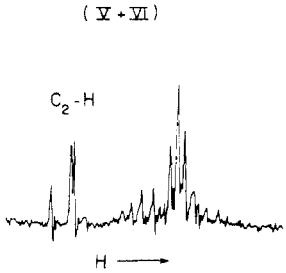


Fig. 1.—The n.m.r. signal for the C_2–H appears as a doublet as expected for a mixture of *cis* and *trans* isomers (V + VI) ($CHCl_3$ as solvent, tetramethylsilane (TMS) as an internal reference).

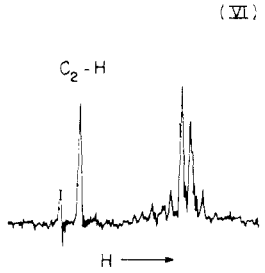
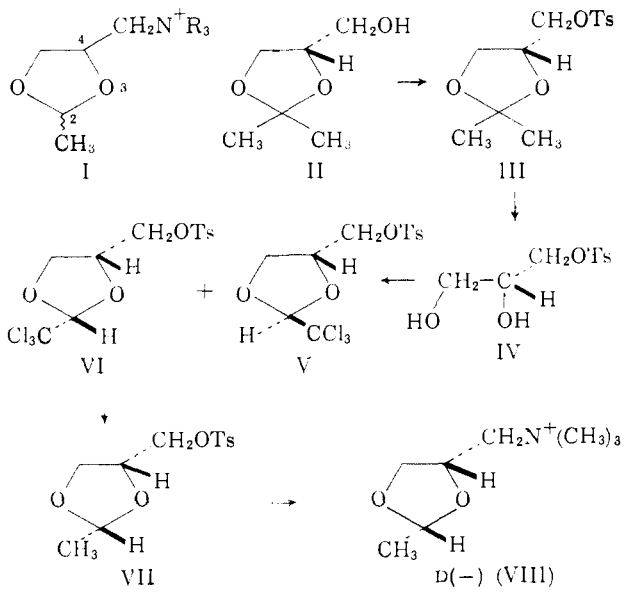


Fig. 2.—The n.m.r. signal for the C_2–H appears as a singlet as expected for pure *cis*-VI ($CHCl_3$ as solvent, TMS as internal reference).

with considerable loss. The composition of the mixture was established by n.m.r. spectroscopy (Fig. 1 and 2). No effort was made to obtain V in pure form. The configuration of VI was established by a comparison of its infrared spectrum with that of the corresponding racemate which is of established configuration.[1] Catalytic hydrogenolysis of VI as previously described[1] for the corresponding racemate afforded VII which when treated with dimethylamine and quaternized finally gave VIII (D-*cis*-F2268), m.p. 144–147°, $[\alpha]_D^{23}$ −16.0°. The homogeneity of this material was ascertained by n.m.r. spectroscopy. As can be seen in Fig. 3, the sharp methyl doublet at high field indicates that the compound must be at least 95% pure. The major impurity, if any is present, could consist of the D-*trans*-isomer. When a mixture of the *dl-cis* and *dl-trans* isomers was analyzed, two high field methyl doublets could be discerned easily (Fig. 4). The discrepancy between the melting points of our preparations and those of Harper, *et al.*,[3] may be attributed either to polymorphism or to the presence in our product of small amounts of an impurity undetectable by n.m.r. or empirical analysis.

CHART I



The synthesis of the L(+) enantiomer was accomplished as shown in Chart II. Mild acid hydrolysis[4] of O-benzyl-D-isopropylidene glycerol gave X which underwent monotosylation to XI. Catalytic hydrogenolysis of the latter led to XII which was carried through the same sequence depicted in Chart I. In this way the enantiomers XIII + XIV, XV and finally XVI were consecutively obtained. This latter [(L(+)-*cis*-F2268)] had m.p. 147–150°, and $[\alpha]_D^{23}$ 17.0°. The homogeneity of the product was also ascertained by n.m.r. spectroscopy and the result suggests (Fig. 5) that this enantiomer must be at least 95% pure.[5]

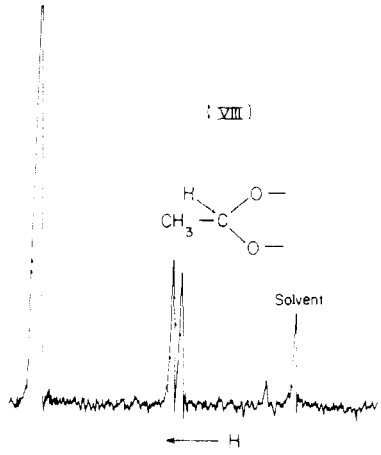


Fig. 3.—The n.m.r. signal for the C_2-methyl group of VIII (pyridine as solvent, TMS as reference) appears as a sharp doublet as expected for the pure *cis* isomer.

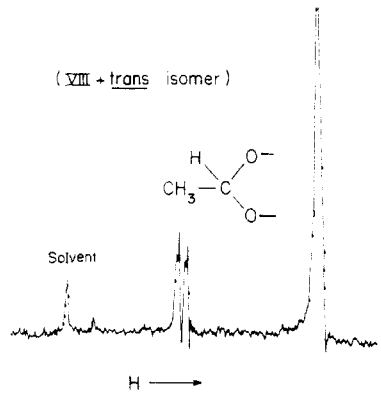


Fig. 4.—The n.m.r. signals for the C_2-methyl groups of a 60:40 mixture of racemic VIII and its *trans* counterpart (see ref. 1) (in pyridine, TMS as reference) appear as two overlapping doublets.

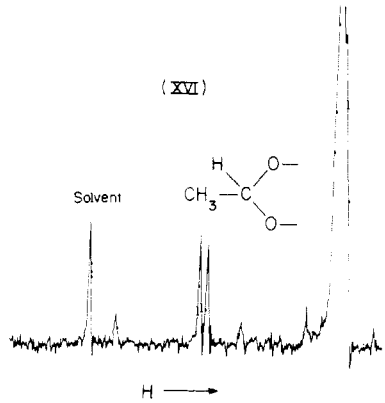


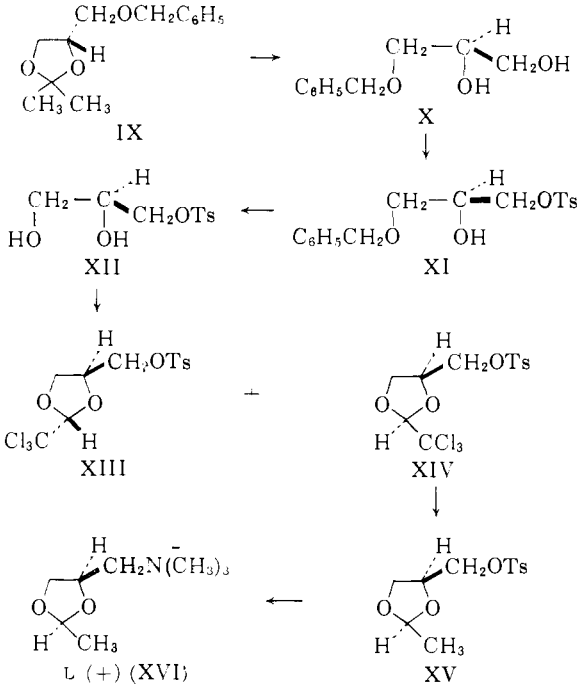
Fig. 5.—The n.m.r. signal for the C_2-methyl group of XVI (in pyridine, TMS as reference) appears as a sharp doublet as expected for the pure *cis* isomer.

(3) N. J. Harper, A. H. Beckett, and R. J. Scott, *Chem. Ind.* (London), 1331 (1962).

(4) J. S. Brimacombe, A. B. Foster, and A. H. Haines, *J. Chem. Soc.*, 2582 (1960).

Pharmacology.[5]—Using the guinea-pig ileum as the test organ, the minimum concentrations of VIII and XVI necessary to elicit a contraction (acetylcholine as standard) were determined. The results were as

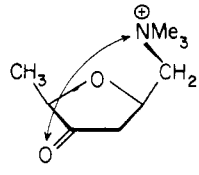
CHART II



follows: (acetylcholine index = 1.0); D(−)(VIII), 0.06; L(+)(XVI) 6.0. The antiacetylcholinesterase activity of these compounds will be reported separately.

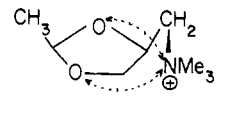
Discussion.—A comprehensive review of structure–action relationships amongst the muscarine group of cholinergic drugs has been published recently by Waser.[6] A particularly puzzling observation is the *inversion* as well as the loss of the optical specificity of the receptors toward muscarone, the D-isomer being more active than its enantiomer, in contrast with the muscarine series where activity is almost an exclusive property of the L-isomers. In order to explain this result, Waser[6] proposed that the pharmacologically active conformation of D-muscarone would be as shown in XVII in which the carbonyl oxygen is considered equivalent in the D-isomer to the ethereal oxygen of the L-isomer. More specifically, Waser postulated that the carbonyl oxygen would now be responsible for hydrogen bonding (with the receptor) and thence for muscarinic activity. This would account for the inversion of the optical specificity since the relative positions of the C2 and C5 substituents with respect to the carbonyl oxygen in D-muscarone and the ethereal oxygen in L-muscarine are enantiomeric. Some serious difficulties with this interpretation lie in that the proposed active conformation (XVII) is of very high energy due to the many unfavorable non-bonded interactions and also in that it departs completely from the maximum coplanarity which is almost uniformly necessary for efficacious binding of the atoms contributing to binding.

We have noted[1] already that the relative stereospecificity of the receptors toward the 1,3-dioxolane series of quaternary salts (I) seemed to parallel that toward the muscarones. If the analogy holds entirely, one would expect the D-isomer VIII to surpass the L-enantiomer XVI in potency since a conformation such as XVIII for the former places the nitrogen at the same distance from the two ethereal oxygens (which are electronically equivalent; this does not really hold for muscarone). This arrangement *although objectionable on several grounds*, would be even more acceptable than conformation XVII for muscarone and would justify better the expectation of an inversion of optical specificity. Since this prediction is not borne out by experiment, the L-isomer being more active by a factor of approx. 100, it follows that conformation XVIII must be ruled out as contributing significantly to activity and the analogy of the dioxolanes to the muscarones is a very restricted one. It would seem also that conformation XVII for D-muscarone is equally unlikely and its greater potency as compared to its enantiomer requires another interpretation.



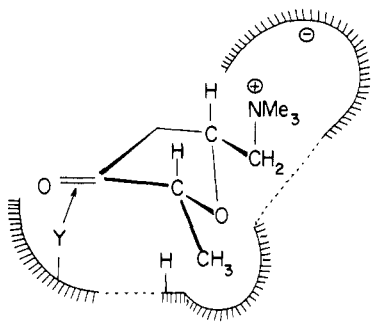
D-Muscarone

XVII

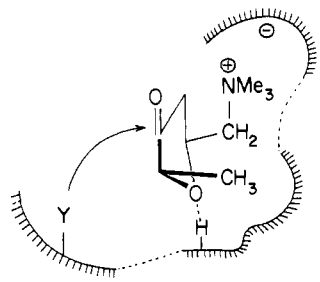


D(−)-cis-F2268

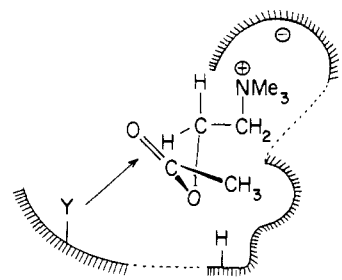
XVIII



XIX



XX



XXIII

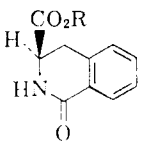
A more likely possibility could be that the carbonyl group in muscarone interacts with an accessory nucleophilic site on the receptor and in this way introduces an additional factor contributing to binding in a unique manner. That the carbonyl group of muscarone might play a special role was recognized by Waser,[6] who stated that "the carbonyl of muscarone has marked polar character which enables it like the electrophilic

(5) The cholinomimetic activities were kindly performed by Dr. M. Pindell and his staff at Bristol Laboratories, Syracuse, N. Y.

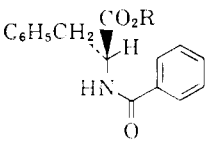
(6) P. G. Waser, *Pharmacol. Rev.*, **13**, 465 (1961).

carbon of an ester to form a covalent bond with some basic group in the receptor." However, this suggestion was not further elaborated. Our results with the enantiomeric dioxolanes VIII and XVI indeed suggest a special role for the carbonyl of muscarone. If a nucleophilic species (Y) would be sterically disposed as shown in the three-dimensional representation XIX, then it can be seen that interaction with the carbonyl group of D-muscarone would contribute additional binding energy. The L-enantiomer in which the carbonyl would be out of reach of the nucleophile Y, as shown in XX, could not benefit from such additional binding energy. The recent demonstration of the presence of the nucleophilic sulfur of methionine in the close vicinity of the active sites of chymotrypsin[7] provides a pertinent precedent for the postulated occurrence of the accessory nucleophilic group (XX). It should be noted that our interpretation clearly accounts also for the non-inversion of the optical specificity toward the dioxolanes.

It is of more than passing interest that the behavior of cholinergic receptors toward the muscarones should find a parallel in the inversion of the optical specificity of chymotrypsin toward the cyclic analog (XXI) of N-benzoylphenylalanine esters (XXII).[8] Interpretations based on conformational analysis of this be-



XXI



XXII

havior of chymotrypsin toward XXI have been proposed.[9,10] It is remarkable that both cases of inversion of optical specificity resulted from the use of cyclic analogs of open-chain substrates. On that basis, there is little doubt that *the mode of binding of* D-*muscarone onto the receptors as shown in XIX must bear a close resemblance to the conformation of receptor-bound acetylcholine for which the conformation XXIII can be derived.* These considerations cannot be reconciled easily with the recent proposals of Archer, Lands and Lewis[11] who extrapolated Waser's rationalizations to the tropine series.

Experimental[12]

D(−)-*cis*-2-Trichloromethyl-4-*p*-toluenesulfonyloxymethyl-1,3-dioxolane (VI).—A mixture of 6.4 g. of D-glycerol monotosylate,[1] 3.8 g. of freshly distilled anhydrous chloral and 2.5 ml. of concd. sulfuric acid was heated slowly to 70° and kept at this temperature for 2 hr. The solution was cooled, poured onto ice-water and the mixture extracted with chloroform. The extract was washed with water, dried and evaporated *in vacuo* and the residue crystallized from methanol to give 4 g. of a white solid m.p. 73–86°. Repeated recrystallization from methanol afforded colorless needles, m.p. 84–85°; $[\alpha]^{23}D$ −6.0° (*c* 2, methanol). The n.m.r. spectrum of this constant melting isomer was compared with that of the mixture (Fig. 1 and 2). As can be seen (Fig. 2), the purified tosylate exhibited only a sharp singlet corresponding to the C_2-hydrogen. The infrared spectrum of this tosylate (in chloroform) was completely superimposable on that of its racemic *cis* counterpart but not on that of its racemic *trans* isomer.[4]

Anal. Calcd. for $C_{12}H_{13}Cl_3O_5S$: C, 38.34; H, 3.46. Found: C, 38.40; H, 3.36.

D(−)-*cis*-2-Methyl-4-trimethylammoniummethyl-1,3-dioxolane Iodide (VIII).—A solution of 1.2 g. of tosylate VI in 35 ml. of ethanol was mixed with a solution of 1.0 g. of sodium bicarbonate in 25 ml. of water and hydrogenated over 0.3 g. of 10% Pd/C under an initial pressure of 2.46 kg./cm.^{2}. After 24 hr., the mixture was filtered and the filtrate concentrated *in vacuo*. The residue was suspended in water and the mixture worked up in the usual manner. The resulting D-*cis*-2-methyl-4-tosyloxymethyl-1,3-dioxolane (VII) was taken up in benzene, the solution saturated with anhydrous dimethylamine and heated to 100° in a pressure bottle for 20 hr. The solvent was removed *in vacuo*, the residue taken up in benzene and the solution shaken vigorously with 2–3 g. of Woelm's alumina (neutral). After filtration, the benzene was evaporated *in vacuo* at room temperature and the residue treated with excess methyl iodide in dry ether. The solid which separated was recrystallized several times from 2-propanol–ethyl acetate to give colorless prisms, m.p. 144–147°; $[\alpha]^{23}D$ −16.0° (*c* 2, methanol). The n.m.r. spectrum of this isomer (in pyridine) is given in Fig. 3. For purposes of comparison, the spectrum of a mixture of *cis*–*trans* isomers is shown in Fig. 4.

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.27. Found: C, 33.26; H, 6.52.

L(+) 1-Benzylglycerol 3-Tosylate (XI).—A quantity of 0.06 mole of 1-benzylglycerol[1] was converted to the 3-tosylate by treatment with a slight excess of tosyl chloride in dry pyridine at 0°. After work up of the mixture in the usual manner, the product was crystallized from ether–hexane; it had m.p. 48°, $[\alpha]^{23}D$ 4.6° (*c* 5, methanol).

Anal. Calcd. for $C_{17}H_{20}SO_5$: C, 60.66; H, 5.99. Found: C, 60.21; H, 5.96.

L(+)-*cis*-2-Trichloromethyl-4-*p*-toluenesulfonyloxymethyl-1,3-dioxolane (XIV).—Benzylglycerol tosylate (XI) was catalytically debenzylated by shaking in methanol over Pd/C under 28.1 kg./cm.^{2} of hydrogen until adsorption of hydrogen ceased. The resulting L-glycerol-1-tosylate (XII) was allowed to react with chloral as described above in the case of the D-enantiomer VI. Repeated recrystallization of the crude product gave XIV, m.p. 84–85°, $[\alpha]^{23}D$ 5.9° (*c* 2.2, methanol).

Anal. Calcd. for $C_{12}H_{13}Cl_3O_5S$: C, 38.34; H, 3.45. Found: C, 38.42; H, 3.41.

L(+)-*cis*-2-Methyl-4-trimethylammoniummethyl-1,3-dioxolane Iodide (XVI).—The same techniques applied to the preparation of VIII from VI were followed. The recrystallized methiodide had m.p. 147–150°; $[\alpha]^{23}D$ +17° (*c* 1.8, methanol). Its homogeneity was ascertained by n.m.r. (Fig. 5). It can be estimated on that basis that this preparation is also of at least 95% purity.

Anal. Calcd. for $C_8H_{18}INO_2$: C, 33.44; H, 6.27. Found: C, 33.28; H, 6.38.

Acknowledgements.—The authors wish to express their gratitude to Bristol Laboratories, Inc., for the financial support of this work.

(7) W. B. Lawson and H. J. Schramm, *J. Am. Chem. Soc.*, **84**, 2015 (1962).

(8) G. Hein, R. B. McGriff, and C. Niemann, *ibid.*, **82**, 1830 (1960).

(9) I. B. Wilson and B. F. Erlanger, *ibid.*, **82**, 6422 (1960).

(10) E. S. Awad, H. Neurath, and B. S. Hartley, *J. Biol. Chem.*, **235**, PC35 (1960).

(11) S. Archer, A. M. Lands, and T. R. Lewis, *J. Med. Pharm. Chem.*, **5**, 423 (1962).

(12) Melting points were determined on a Kofler hot stage and are corrected. The n.m.r. spectra were determined with a Varian instrument operating at 60 Mc. and the infrared spectra with an Infracord instrument. Microanalyses were carried out Mrs. P. Revelle at the University of Ottawa.