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An indirect gas chromatographic determination of the diastereoisomeric composition of pentapiperide methylsulfate

Pentapiperide methylsulfate [4-hydroxy-1,1-dimethylpiperidinium methylsulfate 3-methyl-2-phenylvalerate] (1) is an anticholinergic for the control of hyperchlorhydria and gastrointestinal hypermotility. This substance is a di-chiral compound with the two asymmetric carbon atoms positioned in a *vicinal* configuration. Two diastereoisomeric modifications are possible and, indeed, the synthetic scheme yields an equimolar mixture of the diastereoisomers. Only one of the diastereoisomers — the high melting form — is used in drug formulations. This raw material typically has greater than 97% diastereoisomeric purity as indicated by differential scanning calorimetry (DSC).

An indirect gas chromatographic method for the determination of the diastereoisomeric composition of pentapiperide methylsulfate is presented here. We have found that pentapiperide methylsulfate reacts with lithium aluminum hydride (LAH) in tetrahydrofuran (THF) to yield 2-phenyl-3-methylpentanol (2) (Fig. 1). The separation of the diastereoisomeric modifications of (2) was accomplished by gas chromatography (GC).

A number of papers have appeared describing the analysis of diastereoisomers by GC. GAULT AND FELKIN[1] have separated a number of *erythro* alcohols from the corresponding *threo* alcohols. Publications have appeared describing the separation of diastereoisomeric esters[2–4], amides[3,5,6] and amino acids[7–9]. FEIBUSH AND SPIALTER[10] have reported separation of the diastereoisomers of 2,3-dipentylbutanes and 2,4-dipentylpentanes.

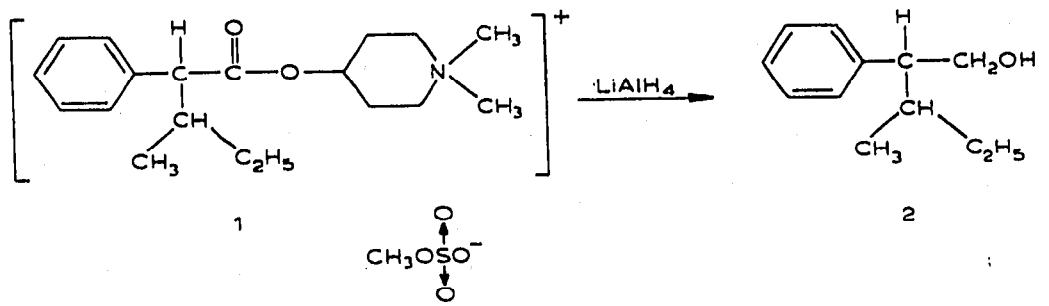

Fig. 1. Reaction of pentapiperide methylsulfate with $LiAlH_4$.

Experimental

Apparatus

A Tracor MT220 gas chromatograph equipped with a dual flame ionization detector was used. The temperature of the injector and detector ports were always maintained at 20–30° above that of the column temperature (see Table I). Gas flow rates were: nitrogen, 25–50 ml/min (flow rate was adjusted to obtain optimum separation); hydrogen, 40 ml/min; air, 390 ml/min. The solid support — Gas-Chrom Q, 80–100 mesh — was purchased from Applied Science Laboratories, State College, Pa. STAP was purchased from Varian Associates, Walnut Creek, Calif. U-shaped Pyrex® glass columns with 1/4 in. O.D. were used.

A Perkin-Elmer Model DSC-1B was used. Melting points were determined on a calibrated Thomas-Hoover Unimelt and are uncorrected. NMR spectra were recorded with a Varian Associates A-60A using tetramethylsilane as the internal standard.

Pentapiperide methylsulfate

This substance consists of two diastereoisomeric modifications. Only the diastereoisomer with m.p. 146–147° could be purified. The purity proved to be 98.6 % by DSC. The purity as indicated by sodium lauryl sulfate titration was 100.5 %. The low melting diastereoisomer could not be obtained in pure form.

2-Phenyl-3-methylpentan-1-ol

This substance was synthesized on a large scale for proof-of-structure work and on a small scale for routine analytical work.

Preparative. A mixture of 3.8 g (0.1 mole) of lithium aluminum hydride and 500 ml of tetrahydrofuran was placed in a three-necked, round-bottomed 1 l flask equipped with a stirrer and condenser. To this mixture was added with vigorous stirring 41.6 g (0.1 mole) of powdered pentapiperide methylsulfate (m.p. 146–147°). The mixture was heated at reflux for 5 min using a steam bath. The flask was then cooled in ice and 150 ml 1 *N* HCl was slowly added (*caution!*). Water (100 ml) was added and the tetrahydrofuran distilled off at atmospheric pressure. The remaining aqueous solution was extracted three times with 100 ml of diethyl ether each time. The ether was dried over anhydrous magnesium sulfate, filtered and evaporated using a rotatory evaporator. The remaining alcohol was vacuum distilled (b.p. 83–85°/0.1 mm), affording 11.74 g (66 % of theory) of 2; NMRδ ($CDCl_3$), 0.70 (doublet, C(3)-CH_3), 0.94 (triplet, C(5)H_3), 1.45 (multiplet, C(3)H, C(4)H_2), 2.46 (quartet, C(2)H), 3.82 (doublet, C(1)H_2), 7.20 (aromatic protons). Anal., calcd. for $C_{12}H_{18}O$: C, 80.85; H, 10.18. Found: C, 80.58; H, 10.12.

Analytical. An accurately weighed sample of about 50 mg of (1) was transferred to a 10 ml centrifuge tube. To this was added 5 ml of dry THF followed by the cautious addition of 200 mg $LiAlH_4$ in small portions. The pentapiperide methylsulfate dissolved with the evolution of gas as it came in contact with the $LiAlH_4$. The solution was then warmed in an oil bath at 50° for 1 h and the mixture was quantitatively transferred to a 50 ml erlenmeyer flask containing 25 ml of anhydrous benzene. This was followed by the dropwise addition of ethanol. The solution was then extracted

TABLE I

GAS CHROMATOGRAPHY OF 2-PHENYL-3-METHYL-1-PENTANOL

Column	*Temperature (°C)*	R^a	α^a	t_R' *(min)*	
				$t_R'_i{}^b$	$t_R'_j{}^b$
6 % SE-30 (4 ft.)	120	0	1.00	26.0	26.0
1 % CW20M (6 ft.)	90	0.921	1.13	15.3	17.3
4 % STAP (6 ft.)	100	1.00	1.11	9.7	10.8
4 % XE-60 (6 ft.)	100	1.07	1.10	45.3	50.0

[a] See *Experimental* for definition of R and α.
[b] Subscripts i and j denote derivatives of the low and high melting isomers respectively.

with three 10 ml portions of 0.1 *N* NaCl. The organic layer was collected in a 50 ml volumetric flask, which contained phenoxyethanol — the internal standard. The solution was diluted to the mark with ethyl acetate and analyzed by gas chromatography (see Table I). The formulation was triturated with chloroform and filtered through Whatman No. 2 filter paper. An aliquot of the filtrate containing 50 ml of (1) was evaporated to dryness under a stream of nitrogen. The residue was reduced and analyzed as described above.

Gas chromatography

The relative volatilities of the two diastereosiomeric alcohols were calculated[6] as follows:

$$\alpha = \frac{t_{Rj} - t_m}{t_{Ri} - t_m}$$

Where t_{Ri} and t_{Rj} represent the uncorrected retention times for the isomers of (2) derived from the low melting and the high melting isomers, respectively. The gas holdup time, t_m, was assumed to be equal to the uncorrected retention time for methane. The resolution $R_{i,j}$ was calculated using the following expression[11]:

$$R_{i,j} = \frac{t_{Ri} - t_{Rj}}{1.669\ (W_{h/2})}$$

Where $W_{h/2}$ is the half-height width of the chromatographic peaks. The results are given in Table I.

Results and discussion

The procedure depicted in Fig. 1 is basically a derivatization technique which must accurately reflect some aspect of the chemical system under study. In this work the aspect which is of greatest interest is diastereoisomeric purity. The two samples of known composition were reduced to the corresponding alcohol mixtures. The result shown in Fig. 2A illustrates that 4-hydroxy-1-methylpiperidine, 3-methyl-2-phenyl-

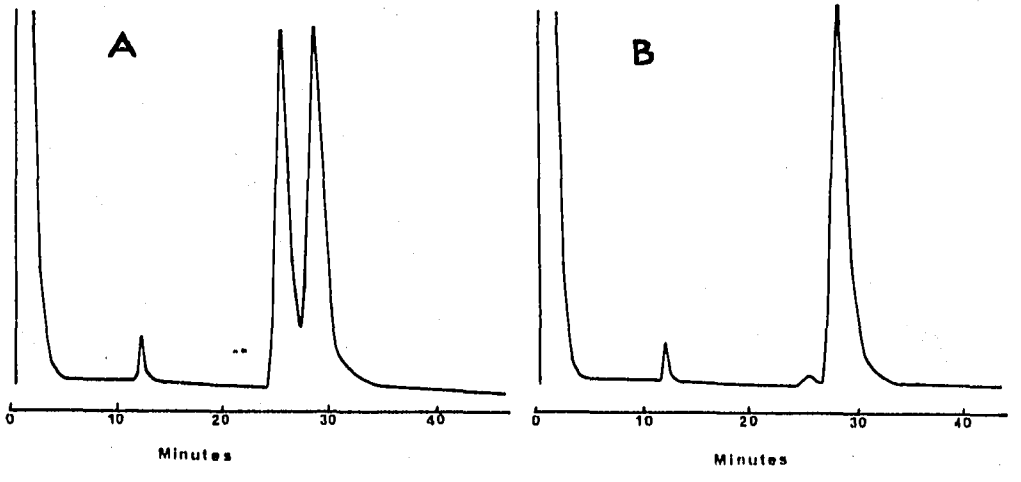

Fig. 2. Chromatogram of (A) the alcohols derived from the reduction of the amine precursor of pentapiperide methylsulfate, and (B) the alcohols derived from pentapiperide methylsulfate, which had a diastereoisomeric purity of 98.6% as determined by DSC. Column: 6 ft. 6% STAP on Gas-Chrom Q, 80/100 mesh at 90°. The small peak at *ca.* 12 min appears in the reagent blank.

valerate, which is an equimolar mixture of two diastereoisomers, yields the expected mixture of alcohols. Fig. 2B shows a chromatogram of the alcohols derived from the reduction of a sample of pentapiperide methylsulfate, which was found to have a diastereoisomeric purity of 98.6 % by DSC. Apparently there is no interconversion of diastereoisomers during the reduction.

Samples were analyzed by GC and sodium lauryl sulfate titration because the two techniques provide complementary data. Hydrolysis of the ester linkage would be readily apparent from the results of the titration and loss of stereochemical purity would be measured by the GC procedure described in this paper.

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