PREFERENTIAL CRYSTALLISATION OF ONE STEREOISOMER FROM SOLUTIONS OF ATROPINE SULPHATE IN ETHANOL

By E. I. Rosenblum and W. S. Taylor

From the Developmental Division, Drug Houses of Australia, Ltd., Melbourne

Received January 13, 1954

The progressive resolution of atropine sulphate during a series of recrystallisations from absolute ethanol was reported in 1928 by Anderson and Hill[1]. Since this did not occur in other solvents, or in ethanolic solutions of atropine alkaloid, atropine oxalate or tropic acid, the authors suggested a specific solubility relationship between the racemic and optically active forms. The present work has shown that atropine and hyoscyamine sulphates crystallise from ethanol with solvent of crystallisation, and that the solubility is considerably less for *l*-hyoscyamine sulphate alcoholate.

When supersaturated solutions of atropine sulphate in ethanol are seeded, the optical activity of the crystals is directed by that of the seeds.

Crystallisation from Ethanol

The atropine sulphate was of zero rotation and m.pt. 194° C., the *l*-hyoscyamine sulphate, $[\alpha]_D$ −27·4° and m.pt. 207° C., and the absolute ethanol contained 0·5 per cent. v/v of water (Karl Fischer titration).

When a 20 per cent. w/v solution of atropine sulphate in hot 99·5 per cent. ethanol is cooled to 20° C. and seeded, 80 per cent. crystallises, and the remainder is dissolved in the mother liquor as a 4 per cent. w/v solution. If saturation is approached at 20° C. by adding the anhydrous sulphate in portions with shaking to the ethanol, it continues to dissolve until approximately 15 per cent. w/v has been added. After a rapid filtration, the solution is clear, but soon begins to deposit crystals of the alcoholate, and, within a short time becomes a magma of crystals in a 4 per cent. w/v mother liquor. The mass of crystals, sucked free from solvent on a Buchner funnel, still contains 60 per cent. of ethanol. This is lost completely in an oven at 50° C., but the rate of drying curve shows a definite fall at a solvent content of 23 to 24 per cent., corresponding fairly closely to $(C_{13}H_{23}O_3N)_2{\cdot}H_2SO_4{,}5C_2H_6O$. The existence of a definite alcoholate is suggested also by a slight rise in temperature which occurs when the anhydrous sulphate is moistened with 99·5 per cent. ethanol.

The behaviour of *l*-hyoscyamine sulphate is entirely similar to that of atropine sulphate, except that both the initial solubility of the anhydrous sulphate and the final solubility of the alcoholate are less. Solubilities of both increase with small additions of water to the solvent, and with increased temperatures, as shown in Tables I and II. In all cases the solubilities were measured after the solution had been in contact with excess crystals for 24 hours.

The marked difference in solubility confirms the suggestion of Anderson

TABLE I

EFFECT OF ADDED WATER ON THE SOLUBILITIES OF ATROPINE AND *l*-HYOSCYAMINE SULPHATE ALCOHOLATES IN ETHANOL

Temperature 13° C.

Ethanol per cent. v/v	Alkaloid sulphate as anhydrous salt	
	Atropine per cent. w/v	*l*-Hyoscyamine per cent. w/v
99·5	2·7	0·8
97·5	6·9	2·1
95·0	19·2	16·8

TABLE II

EFFECT OF TEMPERATURE ON THE SOLUBILITIES OF ATROPINE AND *l*-HYOSCYAMINE SULPHATE ALCOHOLATES IN 99·5 PER CENT. ETHANOL

Temperature ° C.	Alkaloid sulphate as anhydrous salt	
	Atropine per cent. w/v	*l*-Hyoscyamine per cent. w/v
3	1·3	0·4
13	2·7	0·8
20	3·9	1·3
25	5·5	1·6
30	8·4	1·9

and Hill[1] that the spontaneous resolution of atropine sulphate in absolute ethanol is due to a specific solubility relationship.

PREFERENTIAL CRYSTALLISATION

The results of Anderson and Hill[1] were confirmed when a sample of atropine alkaloid of $[\alpha]_D$ −0·3°, dissolved in 99·5 per cent. ethanol, was converted to the sulphate by neutralisation with ethanolic sulphuric acid, and seeded with atropine sulphate of zero rotation. The first crops of crystals in a series of experiments had specific rotations ranging from −1·5° to −5·0°, which increased on one recrystallisation from 20 per cent. w/v solutions in 99·5 per cent. ethanol to an $[\alpha]_D$ from −2·4° to −8·4°. When one sample was submitted to a series of such recrystallisations, the $[\alpha]_D$, as shown in Table III, increased to −19·4° in only 4 steps.

TABLE III

RESOLUTION OF ATROPINE SULPHATE ON RECRYSTALLISATION FROM 99·5 PER CENT. ETHANOL

Seeds with zero rotation added

Step	$[\alpha]_D$ of crystals	Excess of *l*-hyoscyamine sulphate per cent.
Original	−3·9°	14
1	−5·0°	18
2	−8·4°	30
3	−13·9°	50
4	−19·4°	70

Effect of Mixed Solvents

Atropine sulphate of zero rotation was used in a number of experiments on the influence of an added solvent. From 99·5 per cent. ethanol alone, the crystals had $[\alpha]_D$ −2·3°, and the sulphate in solution +4·0°. With 10 per cent. of ethyl acetate added, in no case did the $[\alpha]_D$ of the crystals exceed −0·2°, and from equal parts of ethanol and acetone, the zero rotation was retained.

When a sample of $[\alpha]_D$ −1·6° was recrystallised from ethanol containing 25 per cent. of ethyl acetate by volume, in two experiments the $[\alpha]_D$ of the crystals fell to −0·9° and −0·6°. It is concluded that the second solvent upsets the marked difference in solubility in ethanol alone.

Effect of Rigidly Controlled Seeding

That anhydrous atropine sulphate is a racemic mixture is shown by the melting point curve of its mixtures with *l*-hyoscyamine sulphate, and that the alcoholate is also a racemic mixture, by the observation that *l*-hyoscyamine sulphate alcoholate does not dissolve in a saturated solution of atropine sulphate alcoholate. In testing the directional effect of seeds it was necessary to prevent contamination from the air, since "spontaneous" crystallisation by air-borne seeds is known to give erratic results, and Kipping and Pope[2] have shown that even dust can initiate the resolution of stereoisomers.

20 per cent. w/v solutions of inactive atropine sulphate in hot 99·5 per cent. ethanol were filtered into stoppered flasks, boiled for a few minutes to expel air, and the flasks immediately closed with glass stoppers which had been immersed in ethanol. Such solutions were stable for long periods, and no crystallisation could be induced by shaking, or by cooling to 0° C. When seeded, they crystallised rapidly and completely. With atropine sulphate seeds of zero rotation, the $[\alpha]_D$ of the crystals was −0·3°, with *l*-hyoscyamine sulphate seeds, −6·5°, and with *d*-hyoscyamine seeds, +6·2°.

Summary

1. Atropine and *l*-hyoscyamine sulphates crystallise from ethanol solution as alcoholates.

2. The solubility of *l*-hyoscyamine alcoholate is considerably lower than that of the atropine compound, which may account for the "spontaneous resolution" of the latter.

3. The direction of optical rotation of crystals from deliberately seeded atropine sulphate solutions follows that of the seeds.

The authors are grateful to the Directors of Drug Houses of Australia Ltd. for permission to publish these results.

References

1. Anderson and Hill, *J. chem. Soc.*, 1928, 993.
2. Kipping and Pope, *ibid.*, 1909, 103.