CXXXI.—*A Further Case of the Spontaneous Resolution of Externally Compensated Mixtures.*

By LEONARD ANDERSON and DOUGLAS WILLIAM HILL.

As long ago as 1852 Pasteur (*Ann. Chim.*, **34**, 46) observed that a concentrated solution of inactive ammonium hydrogen malate first deposited, on evaporation, crystals which resembled the active salt in form. This observation was confirmed by van 't Hoff and Dawson (*Ber.*, 1898, **31**, 528), who showed the crystals to consist of a mixture of 3 parts of ammonium hydrogen *l*-malate with 1 part of the *d*-salt, thereby indicating that a partial resolution of the inactive malate had occurred. Malic acid has also been resolved by crystallisation of inactive ammonium molybdomalate (Darmois and Périn, *Compt. rend.*, 1923, **176**, 391). Examples of the spontaneous resolution of sodium ammonium racemate have been recorded by Gernez (*Compt. rend.*, 1866, **63**, 843), Ostromisslensky (*Ber.*, 1908, **41**, 3035), and Kipping and Pope (J., 1909, **95**, 103). Inactive lactic acid has been resolved by Purdie (J., 1893, **63**, 1143), who inoculated concentrated aqueous solutions of its zinc ammonium salt with crystals of the active salt. Werner (*Ber.*, 1914, **47**, 2171) has shown that inactive co-ordination compounds of cobalt and chromium also may be resolved into their active antipodes by similarly inoculating super-saturated aqueous solutions.

The most complete investigation of the effect of inoculation was carried out by Ostromisslensky (*loc. cit.*), who showed that on seeding a *dl*-mixture with an isomorphous substance, one or other antipode is exclusively precipitated. For this purpose he used, besides active tartrates and malates, *l*-asparagine, which caused crystallisation of pure sodium ammonium *d*-tartrate. He also showed that the inoculating crystals need not be optically active or even possess an asymmetric atom.

Such well-defined results, however, have not been obtained by every investigator. For instance, Darmois and Périn (*loc. cit.*) record that inoculation of solutions with a crystal of known sign gives very variable results. Kipping and Pope (*loc. cit.*) too, who at first attributed the crystallisation of sodium ammonium *d*-tartrate from the racemate to the presence of undetected *d*-tartaric acid in the original racemic acid, afterwards drew the conclusion that the presence of dust in the atmosphere was sufficient to cause deposition of either one or other of the isomerides.

In view of the problems which these observations raise, it was considered of interest to put on record a new instance of this phenomenon, namely, the spontaneous resolution of atropine sulphate.

Atropine is prepared commercially by racemisation of the naturally occurring *l*-hyoscyamine and the commercial product is frequently faintly *l*-rotatory. On crystallising this racemised atropine sulphate from absolute alcohol several times, hyoscyamine sulphate of $[\alpha]_D \pm ca.\ 14°$ was obtained regularly, and on further crystallisation of collected crops of this specific rotatory power a hyoscyamine sulphate of $[\alpha]_D \pm ca.\ 20°$ was produced. When the resolution was carried out in a works laboratory in which *l*-hyoscyamine had been racemised, the crop was almost invariably lævorotatory (Table II), but when it was carried out in another laboratory in which the first experiment had shown a predominance of *d*-sulphate in the product of several crystallisations the proportion of crops containing an excess of *l*-hyoscyamine sulphate was considerably decreased (Table III). It is essential to employ a well-dried alcohol for the resolution, and in most cases we have used 99·5% alcohol. In some cases in which the alcohol was not specially dried, little resolution took place (Table IV).

In order to determine whether this resolution of atropine sulphate was due to traces of optically active material present in it or not, a quantity of partly synthetic atropine sulphate of certain inactivity was prepared from synthetic tropic acid and this behaved precisely similarly to atropine sulphate prepared by racemisation of *l*-hyoscyamine, for after five crystallisations from absolute alcohol it gave a product having $[\alpha]_D + 14°$. The results therefore support Kipping and Pope's view that these cases of spontaneous resolution are due, not to undetected traces of an optically active variety, but to inoculation from the atmosphere.

Attempts were also made to effect a separation of atropine sulphate by using a mixture of ethyl acetate and methyl alcohol as solvent, but the original material was unaltered after several crystallisations. Unsuccessful attempts were also made to resolve homatropine sulphate with 99·5% and absolute alcohol as solvents; atropine oxalate, with water and 80% alcohol; atropine alkaloid, with benzene; and tropic acid, with 99·5% alcohol. The specificity of the phenomenon appears to indicate a particular solubility relationship. The final product is different from the original, being much less hygroscopic and less soluble in alcohol. It crystallises well without scratching from five to six times its weight of solvent and does not exhibit the same tendency to form super-saturated solutions —a peculiarity which appears to be common to all the cases of spontaneous resolution so far examined.

EXPERIMENTAL.

Crystallisation of Atropine Sulphate from Alcohol.—Atropine sulphate previously dried at 100° was crystallised from twice its weight of alcohol, and the crystals obtained were collected by means of a pump and immediately recrystallised without drying. Crystallisation, which was on all occasions sluggish, was assisted by cooling in ice-water and vigorous scratching of the super-saturated solution with a glass rod. After several crystallisations this became unnecessary, since the later crops were less soluble and crystallised well on standing in a desiccator. The mother-liquor from each crystallisation was precipitated separately with either acetone or ether, the final crop of crystals and the precipitates being dried at 100° and their rotatory powers in 10% aqueous solution determined.

Experiment A.—Atropine sulphate (25 g.) (m. p. 189—190° and $[\alpha]_D$ — 0·25°) was crystallised as described above six times from absolute alcohol. A final crop (2·1 g.) was obtained with m. p. 201° and $[\alpha]_D$ + 15·43°, corresponding to 59% excess of hyoscyamine sulphate.

Table I gives details of the crops from the mother-liquors.

TABLE I.

Cryst.	Wt. of alcohol used (g.).	Wt. of ppt. from mother-liquor (g.).	M. p. of ppt.	$[\alpha]_D$ of ppt.
A 1	50	8·9	192°	−3·93°
A 2	40	3·9	193	−4·79
A 3	35	3·8	190	+2·50
A 4	30	1·7	192	+1·46
A 5	20	1·2	193	+7·82
A 6	10	0·4	193	—

The results of a number of similar experiments carried out under conditions in which the presence of inoculating particles of the lævo-salt was to be expected are in Table II and of experiments carried out in a separate laboratory in Table III.

The irregular results shown in Table II are due to the presence of varying small amounts of moisture in the alcohol used. In the experiments shown in Table III, only absolute alcohol was employed. The effect of using commercial absolute alcohol which had not been specially dried is shown in Table IV.

Fractional Crystallisation of Atropine Sulphate.—Atropine sulphate (200 g.) previously dried at 100° was dissolved in warm 99·5% alcohol (750 g.) and crystallised under a bell-jar. The fraction (a 1) obtained was recrystallised from 700 g. of alcohol, and the crystallised portion (a 2) again filtered off. Meanwhile, the mother-liquor (M L 1) from the first crystallisation was precipitated by the addition of ether, and this precipitate (b) was crystallised from the

TABLE II.

Exp.	Wt. of atropine sulphate used (g.).	Initial $[\alpha]_D$.	No. of crystns.	$[\alpha]_D$ of final product.	Percentage excess of *l*-hyoscyamine sulphate.
B	25	−3·2°	4	−14·73°	57
C	25	−3·2	5	−14·76	57
D	25	−0·45	6	− 4·60	18
E	25	−0·46	8	−11·50	45
F	50	−0·46	8	−19·50	75
G *	25	−0·40	8	+ 9·25	36
H *	25	−0·80	10	− 8·30	32
I	100	−0·30	10	− 4·75	19
J	25	−4·75	5	−13·40	52
K	25	−0·45	5	−16·85	65
L	300	0·00	12	−16·00	61
M	110	0·00	10	− 9·75	39

* All the experiments were carried out with atropine sulphate of Boots' manufacture with the exception of G and H, in which Boehringer's and Merck's products respectively were used for comparison.

TABLE III.

Exp.	Wt. of atropine sulphate used (g.).	Initial $[\alpha]_D$.	No. of crystns.	$[\alpha]_D$ of final product.	Wt. of final product (g.).	Percentage excess of hyoscyamine sulphate.
N	25	0·00°	5	+14·70°	1·5	57 *d*-
O	25	0·00	7	−13·67	6·2	53 *l*-
P *	20	0·00	5	+14·04	1·4	54 *d*-
A	25	−0·25	6	+15·43	2·1	59 *d*-

* Synthetic atropine sulphate.

Experiment A is included here to make clear the increased proportion of experiments yielding the dextro-salt.

TABLE IV.

Illustrating the effect of moisture.

Exp.	Wt. of atropine sulphate used (g.).	Initial $[\alpha]_D$.	No. of crystns.	$[\alpha]_D$ of final product.
R	60	−0·46°	8	−1·7°
S	30	−0·46	5	−0·7
T	25	−0·45	5	+4·9

filtrate of the second crystallisation, yielding a fresh fraction (b 1), and a fresh mother-liquor (M L 2). This process of crystallising each crop from the preceding filtrate was continued until eight fractions had been obtained. These were dried and their weights and specific rotatory powers are given in Table V. (Determinations of the specific rotatory power were made at different points during the crystallisation, and since fraction b 6 did not crystallise from the mother-liquor of a 7, the value obtained for b 4 is included to indicate the direction of the rotation).

Crystallisation of Synthetic Atropine Sulphate.—The atropine sulphate was obtained by converting synthetic tropic acid, pre-

TABLE V.

Fraction.	M. p.	Wt. (g.).	$[\alpha]_D$.	Percentage of hyoscyamine sulphate.
a 8	200°	18	−13·82°	53 *l*-
(b 4)	—	—	(+10·74)	(41 *d*-)
c 6	200	19	−13·17	51 *l*-
d 5	200	21	+11·48	45 *d*-
e 4	201	35	+11·24	44 *d*-
f 3	200	10	−11·69	46 *l*-
g 2	200	21	−10·35	40 *l*-
h 1	198	15	+ 9·80	38 *d*-
i	191	10	+ 2·86	12 *d*-

pared by Müller's method (*Ber.*, 1918, **51**, 252), into acetyltropyl chloride, condensing this with tropine hydrochloride, and hydrolysing the acetylatropine produced as described by Wolffenstein and Mamlock (*Ber.*, 1908, **41**, 723). The atropine was converted into sulphate and showed no optical activity in a 10% aqueous solution. After five crystallisations from absolute alcohol, the final crop had m. p. 198° and $[\alpha]_D$ + 14·04° (see Table III, Exp. P).

Further Separation of Active Fractions.—(a) The lævorotatory fractions from a number of experiments were collected and dried and 25 g. were crystallised from absolute alcohol in the usual manner. After six crystallisations, the first three each from 150 g. of alcohol and the last three each from 100 g. of alcohol, the final crystallised product (8 g.) had m. p. 206° and $[\alpha]_D$ − 20·58°.

(b) Similarly, 25 g. of the collected dextrorotatory fractions were crystallised each six times from 100 g. of absolute alcohol. The final crop (8·5 g.) had m. p. 207° and $[\alpha]_D$ + 20·12°.

The two rotations recorded correspond to an excess of hyoscyamine sulphate amounting to 79% *l*- and 77% *d*-, respectively.

Specific Rotatory Power of the Free Alkaloid.—The most active fraction obtained in (a) above, having $[\alpha]_D$ − 20·58°, was dissolved in water, and the alkaloid precipitated by sodium hydroxide. The precipitate was well washed with water and dried in a desiccator over sulphuric acid : its specific rotatory power in 10% solution in 90% alcohol was $[\alpha]_D$ − 13·17°.

The same procedure was adopted also in experiment K, the final crystallised product, $[\alpha]_D$ − 16·85°, and the crop from the first mother-liquor, $[\alpha]_D$ + 7·60°, yielding atropine alkaloid with $[\alpha]_D$ − 12·10° and + 5·6°, respectively.

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RESEARCH LABORATORIES, MESSRS. BOOTS PURE DRUG CO., LTD.,
NOTTINGHAM. [*Received, February 14th*, 1928.]