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STUDIES IN THE CONVERSION OF CHAKSINE INTO ITS ISOMERS

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Siddiqui and Ahmad reported the isolation of an alkaloid, chaksine, from the seeds of *Cassia absus* Linn. in 1934, and provisionally assigned to it the formula $C_{12}H_{21}O_2N_3$,[1] which was later revised to $C_{11}H_{21}O_3N_3$ by Ray *et al.*[2] and adopted by Siddiqui and collaborators on the basis of subsequent studies in its structure.[3-5] The first communication on the subject also reported the isolation of an isomeric base of chaksine, *iso*-chaksine (loc. cit.). Both these bases were noted to have a quaternary character, and the authors described the various ways by which chaksine could be converted into *iso*-chaksine. Due to the fact, however, that the salts of *iso*-chaksine, unlike those of chaksine, were not crystallisable, it could not be obtained in a pure form.

While the structure of chaksine has been the subject of numerous studies, and some attention has also been given to the investigation of its pharmacological activity,[6-8] there has been no subsequent reference to the work on *iso*-chaksine. It was, therefore, of considerable interest to re-investigate the position in regard to this isomeric base, with respect to its physiological action and structural relationship with chaksine. Such a study gained added significance, when *iso*-chaksine was found to possess powerful hypotensive activity and much lower toxicity in comparison to chaksine.[9]

In the course of the present studies, a number of discrepancies were noted in respect of procedures described by Siddiqui and Ahmad (loc. cit.) for the conversion of chaksine into *iso*-chaksine, and also unaccountable variations in regard to the hypotensive action of the *iso*-base obtained through these procedures. As a result of the studies described in the 'experimental' with reference to earlier work, it has been found that the conversion of chaksine into *iso*-chaksine has quite a complicated character, and the situation in this regard is summarised as follows.

The behaviour of chaksine bicarbonate on refluxing in alcoholic medium seemed to indicate that, in the process of conversion of chaksine into *iso*-chaksine, an intermediate isomer is formed which is reversible into chaksine in the acidic medium, and gets gradually converted in the basic state into *iso*-chaksine.

Subsequent studies led to the actual isolation of the intermediate isomer which has been provisionally named as *neo*-chaksine. The various ways in which this isomer can be obtained, are described in the 'experimental'. In whichever manner it is prepared, it forms a crystalline bicarbonate melting at 155°, as against the bicarbonate of chaksine m.p. 80°. The bicarbonate is converted into chaksine sulphate on treatment with dilute sulphuric acid, (m.p. 317-19°), but through double decomposition with a solution of sodium sulphate, it gives a crystalline sulphate melting at 255°. When, however, *neo*-chaksine sulphate is re-crystallised through aqueous solution in the hot, the m.p. goes upto 292°, and on acidification it gets wholly converted into chaksine sulphate. It also gave crystalline salts with citric and other organic acids, on keeping the reaction medium distinctly alkaline.

When chaksine bicarbonate is refluxed with alcohol, it takes about 1½ to 2 hours to go into solution, and it is only after about three hours of subsequent refluxing, that 75-80 percent of it is converted into *iso*-chaksine. In this process, some degradation also occurs, as evidenced by the fact that appreciable quantities of ammonia are evolved in the process. The product obtained after 5 hours of total refluxing period was found to be highly hypotensive in action. Occasionally, however, the physiological activity of the refluxed product was very low—about 20 percent. It was noted, moreover, that when the residue left after the removal of the solvent from the refluxed al-

coholic solution is taken up in 1 percent sulphuric acid, it gradually gives a crystallysate, which could be identified with chaksine sulphate, the yield of the salt being about 20 percent of the starting material. When, however, the refluxed base is allowed to stand in alcoholic solution, complete conversion of chaksine into *iso*-chaksine seems to occur, and there is no further formation of crystalline chaksine sulphate on its treatment with sulphuric acid, but the pharmacological activity is reduced by 80 percent.

From the observations briefly recorded above, it would appear that while the reversible isomer *neo*-chaksine is a uniform product, and fairly stable in the alkaline medium, *iso*-chaksine as obtained by any of the processes described by Siddiqui *et al.* or in the present communication, cannot be considered a uniform chemical entity, due to possible admixture of residual *neo*-chaksine, on the one hand, and small quantities of degradation products of chaksine, on the other.

Further studies in *iso*-chaksine, which appear to be justified in view of its powerful hypotensive activity, are in progress, and will be the subject matter of a subsequent communication.

Experimental

20 g. crude chaksine iodide was recrystallised through a mixture of 1:1 methanol and benzene, and then converted into the bicarbonate by treating it with a concentrated solution of sodium bicarbonate in the hot. The resulting product was sucked and crystallised from 3 percent sodium bicarbonate solution. The bicarbonate thus obtained melted at 180°C. (yield 20 g.) and gave a negative test for halogen. It was found important to check upon this, as in some experiments chaksine iodide was not wholly converted into the bicarbonate, and the product had to be re-treated with sodium bicarbonate.

Neo-chaksine.—2 g. of chaksine bicarbonate were introduced into 40 ml. boiling water and refluxed for about two minutes in the course of which it completely went into solution, which was freed of the solvent *in vacuo* at room temperature. It began to crystallise out on concentration and yielded three crops of crystals, the first two of which (needles, Fig. 1) melted at 150°C., yield 1.60 g. On recrystallisation from dilute methanol, it melted at 155°C. Found: C, 49.20; H, 7.53; N, 13.99., required for $C_{11}H_{20}O_2N_3.HCO_3.\frac{1}{2}H_2O$; C, 48.55; H, 7.47; N, 14.15.

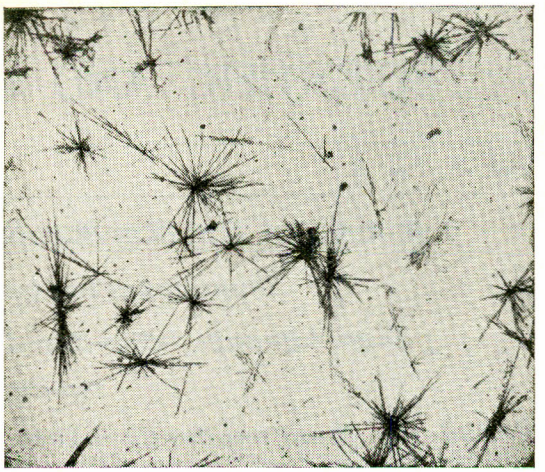

Fig. 1.—*neo*-chaksine bicarbonate.

Another procedure for the conversion of chaksine into *neo*-chaksine consisted in repeatedly treating chaksine sulphate with 5 percent caustic alkali, till the aqueous decantate did not give any test for the sulphate ion, taking up the liberated base in water, passing a vigorous stream of carbon dioxide through the solution and removing the solvent *in vacuo*. In this process, however, a considerable quantity of chaksine is converted into the non-reversible isomer, *iso*-chaksine, which does not give a crystalline sulphate.

Sulphate.—1 g. of *neo*-chaksine bicarbonate was treated with a concentrated solution of sodium sulphate in the hot. The resulting product was sucked, repeatedly washed with water and crystallised from dilute methanol, containing a little sodium acetate to keep the solution distinctly alkaline, in aggregates of needles, m.p. 255°. Found: S, 6.12%; $(C_{11}H_{20}O_2N_3)_2SO_4$ requires S, 5.83%. When the sulphate was crystallised from water, its m.p. went up to 290°, and on crystallisation from dilute sulphuric acid, it gave chaksine sulphate, m.p. 317° (No depression in mixed m.p.).

Oxalate.—It was obtained in the form of stout prismatic rods on treating *neo*-chaksine carbonate with a fairly concentrated aqueous solution of sodium oxalate. When washed and dried, it melted with decomposition at 278-80°, after shrinking and swelling from 200°C. onwards. Crystallisation from 2 percent sodium oxalate solution did not affect the m.p. When prepared by adding 1 percent oxalic acid solution to the carbonate till there was no further effervescence and the pH had come down to 4.5, it melted with decomposition at 290°, apparently due to conversion into chaksine

oxalate (recorded m.p. 290°)[3] in the acidic medium.

Citrate.—It was prepared by adding 1 percent citric acid solution to the *neo*-chaksine bi-carbonate, taking care that the pH remains on the alkaline side and the carbonate is completely decomposed. The citrate thus obtained formed a glistening crystallysate, (net-work of needles) which, when washed and dried on the porous plate, melted at 202° after so ftening and shrinking a few degrees earlier. Recrystallised from 1:1 methanol:water, in which it was fairly soluble, its m.p. was not affected. When crystallised from citric acid, it was apparently converted into chaksine citrate melting at 224-26°. Chaksine citrate prepared by treating the carbonate with citric acid and recrystallising from 1 percent citric acid, showed the same m.p. as against 235°, recorded in the earlier communication.[3]

Succinate.—It was obtained in the form of a net-work of short needles on treating *neo*-chaksine bi-carbonate with a concentrated solution of sodium succinate. It melted at 176°, with previous shrinking and softening as against chaksine succinate m.p. 218°. When recrystallised from 2 percent sodium succinate solution, its m.p. was unchanged.

Melting points of the different *neo*-chaksine and corresponding chaksine-salts are given in Table 1.

TABLE 1.

S. No.	Name of the salt	Chaksine m.p.	*Neo* chaksine m.p.	m.ps. chaksine salts recorded in literature [1,3]
1.	Bicarbonate	180°	155°	180°
2.	Sulphate	317°	255°	317°
3.	Oxalate	290°	278-80°	311°
4.	Citrate	224-26°	202°	235°
5.	Succinate	218°	176°	219°

CONVERSION OF CHAKSINE INTO ISO-CHAKSINE

Siddiqui *et al.* had reported the conversion of chaksine into *iso*-chaksine through treatment of the sulphate of the base with an aqueous solution of barium hydroxide, and also on refluxing an alcoholic solution of chaksine bicarbonate. From the analytical data recorded by them for *iso*-chaksine carbonate, its purity was considered doubtful, and the procedures described for the isomerisation of the base were not fully defined. As a result of the present study it has been shown that the treatment of chaksine sulphate with a solution of barium hydroxide at room temperature fails to effect the conversion, which is brought about only on carrying out this treatment in the hot (water bath), as also on refluxing chaksine bicarbonate in aqueous medium for about half an hour. In both these cases the product obtained did not show any hypotensive activity in contrast to the strong blood pressure reducing action observed with the product obtained according to the isomerisation procedure recorded as follows.

1 g. of chaksine bicarbonate was refluxed with 20 ml. absolute alcohol till it completely went into solution in the course of one hour and a half and the refluxing was continued further for a period of three hours and a half. On removal of solvent *in vacuo*, a cream coloured hygroscopic, fluffy mass was obtained. The optical activity of the product thus obtained ranged in repeated experiments from +41.4 to 49.3 in 1 percent alcoholic solution, and $[\alpha]_D$ of its hydrochloride was +36.4° as against +51.4° of chaksine hydrochloride. When treated with 1 percent sulphuric acid, it gradually deposited crystalline chaksine sulphate in a quantity indicative of the fact (30 mg. from 100 mg. of bicarbonate) that about 25 percent of the reversible isomer *neo*-chaksine still persisted at this stage. When, however, the refluxed alcoholic solution was kept at room temperature for two days, the conversion was complete and no crystalline sulphate could be obtained on treating the residue left on removal of the solvent with diluted sulphuric acid. With this operation, however, the hypotensive activity of the product is reduced to about 20 percent.

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