

then with ether. Removal of light petroleum left an oily residue which on keeping gave a minute quantity of an acid which on several crystallisations from water melted at 116°C. and was identical with the acid described in the subsequent experiment.

Drying and removal of the ether gave material which crystallised from water (m.p. 196°C. dec.) with evolution of gas. Yield 0.12 g. It was identified as phthalic acid with which it gave no depression in melting point. On melting it gave the anhydride which was purified by sublimation. m.p. 130°C., undepressed with an authentic sample of phthalic anhydride. On heating with water gave phthalic acid m.p. 196°C.

**Acid C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>.**—Chaksine iodide (1.0 g.) thoroughly mixed with copper or silver filings (1 g. 40-80 mesh) was heated, in the manner described above, for 25 minutes. Yield of ether soluble red oil with characteristic caraway-like smell varied between 0.2-0.25 g. In one experiment 0.39 g. of the oil was obtained.

The ether soluble oil (0.85 g.) was taken up in 20% methyl alcoholic potassium hydroxide solution (10 ml.) and refluxed for 12 hrs. Methyl alcohol was removed in vacuum, the residue was acidified with dilute hydrochloric acid and repeatedly extracted with light petroleum (60-80°C.). Drying and removal of the solvent yielded an oil (0.18 g.) which crystallised on standing, m.p. 106°C. Crystallisation from

water (twice) gave colourless prismatic needles, m.p. 116°C., (70 mg.). The compound is an acid and is soluble in all the usual organic solvents.

Calculated for C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>: C, 73.17; H, 7.32; O, 19.51; m.w., 164. Found: C, 73.39; H, 7.3; O, 19.38; m.w., 165.5; equivalent wt., 165.

On admixture with an authentic sample of *p*-isopropylbenzoic acid (m.p. 116°C.) the melting point was undepressed.

#### Acknowledgement

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#### References

1. A. Kamal, and G. Hahn, *J. Chem. Soc.*, 555 (1958).
2. S. Siddiqui, G. Hahn, V.N. Sharma and A. Kamal, *Nature*, **178**, 373 (1956).
3. S. Siddiqui, G. Hahn, V.N. Sharma and A. Kamal, *Chem. and Ind. (London)*, 1525 (1956).

### CHAKSINE. PART III

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Acetylation of chaksine furnishes diacetyl-desiminochaksine (II), which accordingly yields desiminochaksine (III) on deacetylation, wherein the guanidine-imino group has been replaced by oxygen. The guanidine group first established in Part I is thus confirmed by conversion to the corresponding urea.

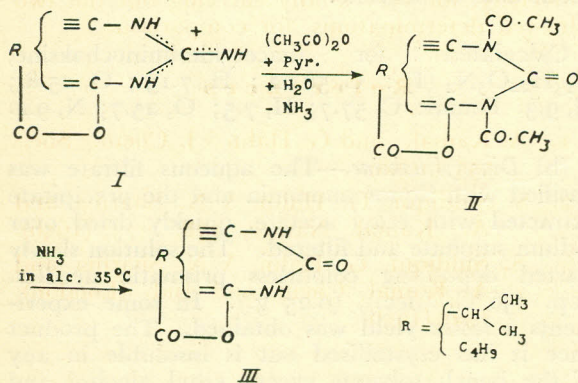
On hydrolysis of chaksine with an excess of aqueous barium hydroxide one molecule of ammonia is evolved within approximate 48 hrs.

while it takes 200 hrs. more to develop another molecule of ammonia together with one molecule of carbon dioxide, leaving behind a water soluble and a difficultly water soluble amino acid, which are under investigation.

Even after one molecule of ammonia has been evolved the product of hydrolysis carries a carboxyl group (m.p. 123°C.). It follows, that besides the replacement of the guanidine-imino group by oxygen a carbonyl group containing, hydro-

lisable grouping of the molecule must have been split off simultaneously. This carbonyl group cannot stand adjacent to one of the imino groups of the guanidine, because it is well-known that such guanidine derivatives drop down considerably in their basicity. In comparison chaksine has still a  $pK_a = 11-12$ . It is therefore suggested that a lactone group is present accounting for the two oxygen atoms in chaksine. Consequently the two acetyl groups in diacetylchaksine must be attached at the two imino groups of the guanidine. On the other hand, the development of two molecules of ammonia and one molecule of carbon-dioxide out of a N,N-disubstituted guanidine is only possible, if the oxy group of the lactone is combined with a carbon atom adjacent to one of the imino groups of the guanidine.

We therefore consider the structure (I) present in chaksine.



The  $C_{10}$ -moiety combined with guanidine makes it obvious that two isoprene molecules might have been involved in the phyto-synthesis of chaksine, so much the more we have isolated *p*-isopropylbenzoic acid by pyrolysis of chaksine iodide with copper filings.<sup>2</sup> An isopropyl group must therefore be present and besides this there might further be a  $-C =$  group, because it is



not sure that the one molecule of acetic acid, received with the Kuhn-Roth oxidation stems from the isopropyl group.<sup>3,4</sup>

As it is possible to put up several monoterpene structures containing the guanidine moiety and explaining all the findings so far, we deliberately withhold to formulate any such suggestion until after more experimental evidence for one or the other is available.

Guha and Ray<sup>5</sup> described the acetylation of chaksine as allegedly resulting in the elimination of an aminoethanol chain, thus furnishing a

"triacetyl compound" of the formula  $C_{15}H_{20}O_5N_2$  m.p.  $200^\circ C$ . without any further confirmation.

According to the established guanidine structure of chaksine one acetyl group could be expected at the nitrogen atom carrying the nitro group in nitrochaksine sulphate. If so this must similarly result in a considerable drop in the basicity and perhaps a corresponding instability as observed with nitrochaksine sulphate. We therefore carried out the same acetylation, isolated the acetyl-product in 25% yield. m.p.  $198-199^\circ C$ . (dil. acetic acid) and found: C, 57.7; H, 7.5; O, 25.7; N, 9.2; which accounts as well for diacetyl-desiminochaksine, wherein the guanidine-imino group has been replaced by oxygen.  $C_{15}H_{22}O_5N_2$  requires: C, 57.9; H, 7.08; O, 25.7; N, 9.05. This seems so much the more obvious, because the oily acetylation product had to be heated for 10 hrs. with dilute acetic acid to become crystalline. In fact the deacetylation product with saturated alcoholic ammonia at room temperature ( $35^\circ C$ .) furnished desiminochaksine, m.p.  $253^\circ C$ . (methanol, dil. acetic acid or water). Calculated for  $C_{11}H_{18}O_3N_2$ : C, 58.3; H, 7.95; O, 21.2; N, 12.3. Found: C, 58.66; H, 7.97; O, 21.57; N, 12.26. According to the urea structure, concentrated nitric acid forms at once the well crystallising nitrate which however on standing with water hydrolyses, furnishing back the starting desiminochaksine. On heating, the nitrate undergoes interesting changes still under investigation.

On carefully basifying the mother liquors of the acetylation, the diacetylchaksine m.p.  $256^\circ C$ . (dec.) could be isolated. It is unstable like nitrochaksine and easily undergoes hydrolysis. It therefore could not be purified reliably. Calculated for  $C_{15}H_{23}O_4N_3$ : C, 58.20; H, 7.5; O, 20.69; N, 13.58. Found: C, 57.31; H, 7.89; O, 19.62; N, 14.66. It is no more basic enough to form the guanidinium hydroxide.

The same drop in the basicity could be observed with nitrochaksine. Out of the basified mother liquors of the nitrochaksine preparation the free nitrochaksine similarly was found. Globulous clusters m.p.  $166^\circ C$ . (methanol). Calculated for  $C_{11}H_{18}O_4N_4$ : C, 48.8; H, 6.6; O, 23.7; N, 20.7. Found: C, 49.02; H, 6.85; O, 23.7; N, 20.4.

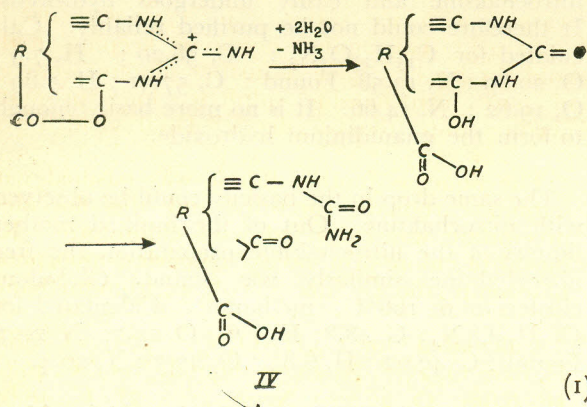
In confirmation of that sulphuric acid furnished nitrochaksine sulphate and on heating with dilute sulphuric acid the same hydrolysis to nitrourea-chaksine takes place; as observed in the case of nitrochaksine.

Most probably the more unstable nitro-

chaksinium hydroxide could also be isolated. On repeated recrystallisation out of 16% alcohol the base m.p. 166°C. was more and more transferred into a base, difficultly soluble in alcohol and melting at 190°C. This base being amorphous and not so far recrystallisable could also be converted into nitrochaksine sulphate and subsequently into nitroureachaksine. Dissolved in chloroform and kept several days over dry sodium sulphate the base m.p. 166°C. could be recovered.

According to these findings we propose to use the name chaksine for the free guanidine, while the hydroxide and its salts should be considered chaksinium compounds.

On heating chaksine with aqueous barium hydroxide in the apparatus described in Part I<sup>1</sup> until one molecule of ammonia has evolved and removing the excess of barium hydroxide with carbon dioxide, a barium salt solution is obtained. This barium salt is highly hygroscopic and its acid, set free with ammonium sulphate and barium carbonate, is also hygroscopic and easily soluble in water. So far only a very small amount of that acid could be isolated. It melts at 123°C. and is under investigation. Since the necessarily present carbonyl group cannot be connected with an imino group of the guanidine, considering the strong basic character of chaksine, the remaining possibility is a lactone group where the oxy group must be connected with the carbon atom adjacent to one of the imino groups of the guanidine. The barium hydroxide hydrolysis can then be understood according to equation (1).



Besides hydrolysis of the imino group of the guanidine, furnishing the first molecule of ammonia, the lactone ring opens and an ureido acid IV is formed which might take 200 hrs. more for being hydrolysed further since it is well-known

that urea itself requires extreme conditions (200°C. and a long time) to be hydrolysed by barium hydroxide.<sup>6</sup>

## Experimental

**Acetylation of Chaksine Sulphate.** (a) *Diacetyl-desiminochaksine* (II).—Chaksine sulphate (5 g.) in acetic anhydride (50 ml.) containing pyridine (0.5 ml.) was heated on water-bath (2 hrs.). Excess of acetic anhydride (ca. 45 ml.) was removed (vac.), and water (200 ml.) added. The contents were heated on water-bath (10 hrs.), cooled and the crystalline acetylated product filtered off. The diacetyl-desiminochaksine was obtained as colourless prismatic needles. m.p. 194°C. (1.43 g; 25.5%). On crystallisation from dilute acetic acid gave the product m.p. 198-199°C. Shows no optical rotation in glacial acetic acid or benzene.

Calculated for diacetyl-desiminochaksine,  $\text{C}_{15}\text{H}_{22}\text{O}_5\text{N}_2$  (I): C, 58.02; H, 7.15; O, 25.8; N, 9.3. Found: C, 57.7; H, 7.5; O, 25.7; N, 9.2.

(b) *Diacetylchaksine*.—The aqueous filtrate was basified with liquor ammonia and the precipitate extracted with ethyl acetate, quickly dried over sodium sulphate and filtered. The solution slowly started depositing colourless prismatic needles. m.p. 256°C. (dec.), (0.25 g.). In some experiments a lesser yield was obtained. The product once it has crystallised out is insoluble in any of the bench solvents except ethyl alcohol and tetrahydrofuran in which it dissolves sparingly, a lacquer-like deposit being obtained on removal of the solvents. It could not therefore be purified properly.

Calculated for diacetylchaksine  $\text{C}_{15}\text{H}_{23}\text{O}_4\text{N}_3$ : C, 58.2; H, 7.5; O, 20.7; N, 12.58. Found: C, 57.3; H, 7.9; O, 19.6; N, 14.6.

**Derivative of Diacetylchaksine.** *Diacetylchaksine Nitrate*: from diacetylchaksine (0.1 g.) and dilute nitric acid. Crystallised from water. Prisms. m.p. 187°C. (dec.)  $[\alpha]_{\text{D}}^{29.5} + 74.87$  in water.

(I) Calculated for diacetylchaksine nitrate:  $\text{C}_{15}\text{H}_{24}\text{O}_7\text{N}_4$ : C, 48.4; H, 6.45; O, 30.11; N, 15.1. Found: C, 48.9; H, 6.81; O, 28.25; N, 15.5.

Removal of ethyl acetate from the mother-liquor after removal of diacetylchaksine gave an almost colourless weak amorphous base (A) (1.2 g.) soluble in ethyl acetate and methyl and

ethyl alcohols. It formed salts with mineral acids which hydrolyse on dilution with water.

**Acetylation of Amorphous Base (A).**—Amorphous base (A), (0.5 g.), taken up in acetic anhydride (5 ml.) containing a drop of pyridine was heated for 2 hrs. worked up as in the acetylation of chaksine sulphate. Colourless prisms m.p. 182°C., (0.16 g.), recrystallised from dilute acetic acid m.p. 198°C. undepressed with diacetyl-desiminochaksine (II).

**Acetylation of Chaksine Nitrate.**—Chaksine nitrate (0.4 g.) in acetic anhydride (6 ml.) containing pyridine (2 drops) was refluxed on sand bath (5 minutes). Solution took place with evolution of oxides of nitrogen. Poured into water (80 ml.) and allowed to stand (24 hrs.). Residue crystallised from dilute acetic acid, prismatic needles, m.p. 197-198°C. (0.13 g.), undepressed on admixture with diacetyl-desiminochaksine (II). Shows no optical rotation in acetic acid or benzene.

**Acetylation of Base 256°C. (Diacetylchaksine).**—Base 256°C. (0.1 g.) in acetic anhydride (1 ml.) containing pyridine (1 drop), heated on water bath (2 hrs.). Removed acetic anhydride in vacuum, added water (5 ml.), heated on water-bath (10 hrs.) and the colourless crystalline product collected. m.p. 187-188°C. Recrystallised from dilute acetic acid m.p. 198-199°C., undepressed with diacetyl-desiminochaksine (II).

**Desiminochaksine (III).**—(a) *Deacetylation of diacetyl-desiminochaksine with methyl alcoholic ammonia:* Diacetyl-desiminochaksine (I) (4.7 g.) was taken up in methyl alcohol saturated with ammonia, (600 ml.) and kept at about 30°C. for 36 hrs. Removal of solvent from the clear solution gave diacetyl-desiminochaksine as an oil which solidified on standing. Colourless prisms from hot water, dilute methyl alcohol or dilute acetic acid., m.p. 253°C., (1.8 g.).  $[\alpha]_D^{34} + 72.76$  in glacial acetic acid.

Calculated for desiminochaksine (III),  $C_{11}H_{18}O_3N_2$ : C, 58.3; H, 7.95; O, 21.2; N, 12.3. Found: C, 58.66; H, 7.97; O, 21.57; N, 12.26.

(b) *Deacetylation of diacetyl-desiminochaksine with ethyl alcoholic HCl:* Diacetyl-desiminochaksine (0.1 g.) taken in ethanolic HCl (1.8%; 7 ml.) was refluxed for 10 hrs. Removed alcohol in vacuum and the oily residue extracted with hot water (10 ml.), cooled and filtered. Crystals after 7 days; m.p. 242°C. (10 mg.). Recrystallised from hot water; m.p. 253°C. undepressed with desiminochaksine (III).

Found: C, 58.59; H, 8.02; O, 21.48; N, 12.16.

**Acetylation of Desiminochaksine (III).**—Desiminochaksine (III) (80 m.g.) taken up in acetic anhydride (3 ml.) containing a drop of pyridine was refluxed for 3 hrs. Removed acetic anhydride in vacuum and the residue crystallised from dilute acetic acid. Prismatic needles, (50 mg.), m.p. 198°C., undepressed on admixture with diacetyl-desiminochaksine (II).

**Desiminochaksine Nitrate.**—Desiminochaksine (0.2 g.) was treated under ice-cooling, with nitric acid (d. 1.4; 5 drops). Prisms of the nitrate start coming out immediately. After standing (5 minutes) the crystallisate was filtered and pressed on a porous plate to remove the excess of nitric acid and kept in vacuum over sodium hydroxide. m.p. 133°C. (dec.); (0.15 g.).

Calculated for  $C_{11}H_{18}O_3N_3 \cdot HNO_3$ : N, 18.48. Found: N, 18.11.

**Hydrolysis of Desiminochaksine Nitrate.**—Desiminochaksine nitrate (0.1 g.) was covered with a little water and allowed to stand (3-4 hrs.), filtered and crystallised from dilute acetic acid (0.08 g.) m.p. 252°C. Mixed m.p. with desiminochaksine gave no depression.

**Nitrochaksine Sulphate.**—(*Isolation of free nitrochaksine*): Chaksine nitrate (5 g.) was added in small portions and with rapid stirring to sulphuric acid (d. 1.84; 10 ml.), kept between -5°C. to 0°C., till clear solution was obtained. The contents, allowed to stand (45 minutes) at room temperature, were poured into ice (300 g.). Nitrochaksine sulphate, collected by suction, was washed with ice water m.p. 174-6°C. (dec.); (3.51 g.). The mother liquor on keeping in the cold for 24 hrs. gave a further amount (0.8 g.) of pure nitrochaksine sulphate, long prismatic needles. m.p. 180°C. (dec.). Total yield 4.3 g.

The aqueous mother liquor was basified with sodium carbonate carefully and the flocculent precipitate was extracted with chloroform. Removal of chloroform gave a colourless amorphous base. m.p. 166°C. (dec.) (1.12 g.) from methanol.

Calculated for  $C_{11}H_{18}O_4N_4$ , (270): C, 48.8; H, 6.6; O, 23.7; N, 20.7. Found: (1) C, 49.02; H, 6.85; O, 23.73; N, 20.47; (2) C, 48.87; H, 6.62; O, 23.90; N, 20.62.

Repeated crystallisation of the base 166°C. (dec.) from 96% ethyl alcohol changes the substance more and more into a difficultly soluble and unstable base melting at 190°C. (dec.) which

could not be properly recrystallised. Its solution in dry chloroform, on standing over sodium sulphate for a number of days, slowly gives back the original 166°C. (dec.) base.

The base which we believe to be the chaksinium hydroxide of the free chaksine base was characterised through its salts which were, as expected, identical with corresponding salts of the base m.p. 166°C. (dec.).

In view of the identity of the salts of the bases 190°C. (dec.) and 166°C. (dec.) the preparation of the salts of the former alone are being reported.

**Sulphate of Base 190°C. (dec.)** (*Nitrochaksine Sulphate*): The base (0.2 g.) was dissolved in portions in conc. sulphuric acid (d. 1.84; 0.5 ml.), maintained at -5°C., with constant stirring and rubbing, (1.5 hr.). Poured into ice (15 g.) and the product collected was washed with ice-cold water; m.p. 162-164°C. (dec.); (0.16 g.) mixed m.p. with nitrochaksine sulphate 172°C. (dec.).

Since the sulphate decomposes it was not purified by crystallisation from water but was hydrolysed to nitroureachaksine.

*Nitrochaksine Oxalate*.—Base 190°C. (dec.) (0.2 g.) in dioxane (2 ml.) added to dioxane solution of oxalic acid. Globules of nitrochaksine oxalate; m.p. 191°C. (dec.) (0.2 g.)

Calculated for nitrochaksine oxalate  $C_{13}H_{20}O_2N_4$ : C, 43.33; H, 5.55; O, 35.55; N, 15.55. Found: C, 43.51; H, 5.87; O, 35.72; N, 14.97.

*Nitrochaksine Chloride*.—Base 190°C. (dec.), (0.2 g.), dissolved by warming in concentrated hydrochloric acid (1 ml.). Cooled and poured into water. Colours at 200°C. m.p. 215°C. (dec.).

Calculated for nitrochaksine chloride  $C_{11}H_{19}O_4N_4Cl$ : C, 43.05; H, 6.25; O, 20.88; N, 18.27; Cl, 11.5. Found: C, 42.97; H, 6.59; O, 21.10; N, 18.39; Cl, 11.13.

Solution of the chloride in water on basification with ammonia gave the original base m.p. 190°C. (dec.), undepressed on admixture with the starting material.

**Hydrolysis of Sulphate of Base 190°C. (dec.)**.—The sulphate (0.43 g.) taken in water (43 ml.) was heated on water bath. After ½ hr. pH was 4.8 which came down to 2.5 after 8-10 hrs. After 36 hrs. heating was stopped and the crystalline product collected (0.19 g.); m.p. 200°C. (dec.), undepressed with nitroureachaksine.<sup>1</sup>

#### **Direct Hydrolysis of the Base 190°C. (dec.)**

The base (0.3 g.) suspended in water (30 ml.) containing 2 drops of 10% sulphuric acid was heated on water bath. The solution which had pH 1 at the start became clear (½ hr.) and had pH 3-4 after 13½ hrs. After 36 hrs. (pH 2.5) the reaction was stopped and the colourless crystalline material collected. (0.13 g., 41.9%), m.p. 199-200°C. (dec.) undepressed with nitroureachaksine.<sup>1</sup>

#### **Reaction of Nitrochaksine Sulphate with Sodium Carbonate**

—Nitrochaksine sulphate (1.06 g.) was taken up in a solution of sodium carbonate (5.3 g. in 35 ml. water) by warming for ½ hr. at 50-70°C. After standing for 3 hrs. the solution was partly acidified with HCl, flocculent matter (0.1 g.) filtered off and the mother liquor further acidified with HCl (pH 2). A further quantity of a white flocculent precipitate (0.3 g.) was filtered off; m.p. 160-170°C. (frothing). This material is soluble in ethyl and methyl alcohols. With water gets oily on warming and forms a barium salt. Partly soluble in acetone, the acetone insoluble portion being soluble in acetic acid and hot methyl alcohol, and forms an insoluble barium salt. The two fractions are under investigation.

The filtrate now on being neutralised with sodium carbonate (pH 8-9) gave a white flocculent precipitate which crystallised as globules from dioxane and ethyl alcohol (40 mg.). Turns brownish red at 160°C. melting at 166°C. (dec.). Mixed m.p. with the base 166°C. (dec.) (obtained in the reaction of chaksine nitrate and sulphuric acid) undepressed. It is most probably free nitrochaksine.

#### **Acknowledgement**

Micro-analysis was carried out by Dr. A. Bernhardt, Microanalytical Laboratorium, 22A, Mulheim, Ruhr, West Germany.

#### **References**

1. Ahmad Kamal and George Hahn, *Chaksine Part I*, J. Chem. Soc., 555 (1958).
2. S. Siddiqui, George Hahn, A. Kamal and V.N. Sharma, J. Soc. Chem. Ind. (London), 1525 (1956).
3. Richard Kuh and Hubert Rath, Ber., **66**, 1274 (1933).n
4. W.F. Barthel and F.W. LaForge, Ind. Eng. Chem. (Anal. Ed.), **16**, 434 (1944).
5. S.K. Guha and J.N. Ray, J. Indian Chem. Soc. **33** (4), 225.
6. E.A. Werner, J. Chem. Soc., **113**, 84 (1918)