108. *The Isolation of* L-*Stachydrine from the Fruit of* Capparis tomentosa.

By J. W. Cornforth and Alan J. Henry.

L-Stachydrine has been isolated in substantial amount from the pericarp, endocarp, and seed husk of the fruit of *Capparis tomentosa* Lam.

In view of the reputed toxicity to camels of *Capparis tomentosa* Lam. (family Capparidacæ) the fruits of the shrub have been examined for the presence of basic constituents analogous to those previously found (Henry, *Brit. J. Pharmacol.*, 1948, **3**, 187; Henry and Grindley, *J. Soc. Chem. Ind.*, 1949, **68**, 9; Henry and King, *J.*, 1950, 2866; Cornforth and Henry, preceding paper) in the various parts of *Courbonia virgata* A. Brongn., of the same family. *Capparis tomentosa* Lam. is a climbing shrub, the dry, mature fruit of which consists of a hard spherical pericarp 1—2½ inches in diameter, containing a friable pulpy endocarp in which is embedded a number of seeds consisting of a kernel about 10 × 8 × 5 mm. each in a separate seed husk (testa). The husks and pulp were examined separately, during the course of which no evidence could be found for the presence of volatile amines, extractable alkaloids, tetramethylammonium hydroxide or 3-hydroxystachydrine-*a* or -*b*. However, purified extracts gave a heavy precipitate of globules on treatment with iodine solution. A number of periodide fractions were obtained by stepwise precipitation, first in neutral and then in acid solution. All these, however, proved to be of similar nature and to consist essentially of L-stachydrine periodide.

L-Stachydrine has been isolated as both hydrochloride and picrate in high purity, and the constants found for these salts are perhaps more accurate than previously published data. The sample obtained by Steenbock (*J. Biol. Chem.*, 1918, **35**, 1) was evidently partly racemised, perhaps by the barium hydroxide used in the isolation process. On the other hand the data of Yoshimura and Trier (*Z. physiol. Chem.*, 1912, **77**, 301) agree well with our own, except that the rotation of their hydrochloride and the melting point of their picrate are somewhat lower. A synthetic specimen of L-stachydrine hydrochloride was prepared from L-proline, silver oxide, and methyl iodide in methanol. This method was chosen as being unlikely to induce racemisation. The product corresponded in melting point and optical rotation to the specimen from *Capparis tomentosa*.

From the pericarp material, L-stachydrine hydrate was isolated in considerable quantity, purification through a salt being here unnecessary. Some physical properties of this substance are reported for the first time.

Experimental

Extraction of Seed Husk and Pulp.—Husk (500 g.) and pulp (1000 g.) materials, separated as completely as possible from each other, were dried and ground, then twice digested overnight in the cold with ethanol with occasional shaking. The combined ethanol extracts were evaporated at atmospheric temperature with an intermediate filtration to remove separated fat, then diluted with a little water and exhaustively extracted with light petroleum. The aqueous solutions were evaporated at atmospheric temperature to thick syrups, but from neither of them did crystals separate, suggesting absence of the 3-hydroxystachydrines isolated from the husks of *Courbonia virgata*. After suitable dilution the extracts, which were acid in reaction,

were exhaustively extracted with chloroform–ether, then made just alkaline with sodium carbonate and again extracted with chloroform–ether. These operations showed absence of volatile amines and of extractable alkaloids. The extracts were next treated with neutral lead acetate until the solutions became acid followed by basic lead acetate until there was no further precipitation, and excess of lead was removed from the filtrates with hydrogen sulphide. The extracts were again evaporated to thick syrups, but no crystallisation occurred.

Precipitation of Periodides.—The extracts were diluted to 200 c.c. after neutralisation with excess of calcium carbonate and filtration of the excess, and 150 c.c. of each solution treated in distillation flasks of 300 c.c. capacity with iodine solution (9% iodine in 10% ammonium iodide, 50 c.c.) at atmospheric temperature. The solutions were refrigerated for 4 days by which time the precipitated globules of periodide had largely crystallised. Chloroform was added to the flasks through a long-stemmed funnel until the whole of the mother-liquor had been floated out through the side-tube of the flask. After decantation of the chloroform the periodide residues were treated with ethanol (2 c.c.), then with successive small quantities of amyl alcohol until the periodide had only low solubility in it. The periodide residues were transferred to sintered-glass funnels, washed with amyl alcohol, and air-dried. The pulp extract yielded 2·92 g. of solid periodide and the husk extract yielded 3·18 g., m. p. (of both) 230° (decomp.). These periodides were decomposed to hydriodides by treatment with water, then converted into hydrochlorides by being shaken with the freshly precipitated silver chloride. The hydrochloride solutions were evaporated to dryness, dissolved in absolute ethanol, and filtered, then the hydrochlorides recrystallised from water after removal of the ethanol. M. p. (220°) and mixed m. p. showed the two hydrochlorides to be identical. The amyl alcohol wash-liquors were washed with water in the hope of freeing them from ammonium salts and other impurities, but it was found that the L-stachydrine hydriodide was extracted by the water more readily than was ammonium iodide.

The mother-liquors from the first periodide precipitates were further treated with three successive quantities (50, 50, and 100 c.c.) of iodine solution. The periodide fractions so obtained proved to be essentially similar to the first periodide crops, though they tended to become more fluid and more soluble in amyl alcohol, probably as a result of the greater excess of iodine tending to precipitate a higher periodide. Crystals of periodide formed with a limited amount of iodine have been found to become fluid on addition of excess of free iodine.

After separation of the fourth crops of periodide from the neutral solutions, these were acidified by addition of concentrated hydrochloric acid (20 c.c.). Each solution yielded a substantial precipitate of fluid periodide which was isolated as before. These periodides were reduced to hydriodides, first by treatment with water, then by being shaken with silver powder, and the hydriodides converted into hydrochlorides by being shaken with excess of silver chloride. Preliminary examination having shown that the hydrochlorides from the two solutions were identical, these were combined and evaporated to very low bulk and the crystallised hydrochloride was isolated; it had m. p. 220° alone or mixed with the hydrochlorides described above. A further crop of crystals also proved to be identical with the first crop, and the residual mother-liquor on suitable treatment with picric acid yielded a substantial crop of a picrate which was identical with that prepared from the hydrochloride.

Properties of Hydrochloride and Picrate.—Hydrochloride fractions of good quality from both neutral and acid precipitation were combined (6 g.) and recrystallised from hot absolute ethanol. The crystals, stout needles $\frac{1}{2}$—1 inch long, were washed with cold 1 : 1 ethanol–acetone and oven-dried to constant weight. They were anhydrous and non-hygroscopic. The yield was 3·85 g., the m. p. 222° with rapid evolution of gas (Found: C, 46·8; H, 7·9; N, 7·9. Calc. for $C_7H_{14}O_2NCl$: C, 46·8; H, 7·8; N, 7·8%), and $[\alpha]_D^{20}$ was −28·1° (*c*, 4·82 in water) which is significantly higher than previously published figures (Yoshimura and Trier, *loc. cit.*, give $[\alpha]_D^{18}$ −26·2°). A further quantity (1·65 g.) was obtained from the mother-liquor.

The picrate could be prepared by addition of saturated aqueous picric acid solution to a solution of the hydrochloride and evaporation to smaller bulk at atmospheric temperature. It crystallised from water in stout hexagonal prisms, m.p. 199—200° without appreciable decomposition, since the solidified melt remelted at 199—200°.

Synthesis of L-*Stachydrine Hydrochloride.*—L-Proline (1 g.) was added to a suspension of silver oxide (2 g.) in methanol (20 c.c.). When formation of a silver compound appeared complete, methyl iodide (2 c.c.) was added. Evolution of heat and separation of silver iodide were noticed at once. Next day a little more methyl iodide was added and the mixture heated for 3 hours (reflux). The neutral solution was filtered and evaporated at low pressure. To the crystalline residue concentrated hydrochloric acid (1 c.c.) was added. Water was removed by

evaporation with ethanol. The product after two recrystallisations from ethanol gave handsome prisms, m. p. 222° (decomp.) alone or mixed with the above hydrochloride, $[\alpha]_D^{22}$ −27·5° (*c*, 2 in water) (Found: C, 46·8; H, 7·6; N, 7·9%).

Racemisation of the C. tomentosa *Product.*—To confirm the identity of this product with L-stachydrine hydrochloride a sample (241 mg.) was boiled in water (20 c.c.) with barium hydroxide (1 g.) for 5 hours. The barium was precipitated with sulphuric acid and the filtered solution, which was optically inactive, evaporated. From the residue a picrate (yellow plates, m. p. 195—196°, from ethanol) and an oxalate (needles, m. p. 105—107°, from ethanol) were prepared. These correspond to the DL-stachydrine derivatives reported by Schulze and Trier (*Z. physiol. Chem.*, 1910, **67**, 73). The racemisation with barium hydroxide is mentioned by Yoshimura and Trier (*loc. cit.*).

Extraction of Pericarp.—Dry, ground pericarp material (1 kg.) was exhaustively extracted with cold alcohol. The solvent was removed at room temperature and the residue mixed with water. After removal of fat and treatment with lead acetate and hydrogen sulphide as described above, the aqueous solution was allowed to evaporate, finally *in vacuo* over calcium oxide. After some months the partly crystalline mixture (34 g.) was filtered, leaving a solid (5·7 g.) (A) which was purified by crystallisation from water (three crops, total 3·3 g.), followed by dissolution in a little ethanol and addition of chloroform (200 c.c.). The chloroform solution, after decantation from a little resin, was evaporated. The residue on recrystallisation from cold ethanol gave impure material (1·09 g.). By repeated extraction of the evaporated ethanolic mother-liquors with hot chloroform and evaporation to small bulk, L-stachydrine hydrate (1·56 g.) was obtained in hexagonal crystals, m. p. 116—118° (partial melting; in bath at 112°), $[\alpha]_D^{24\cdot5}$ −40·25° (*c*, 4 in water) (Found: loss *in vacuo* over sulphuric acid, 11·4; N, 8·2. Calc. for $C_7H_{13}O_2N,H_2O$: H_2O, 11·2; N, 8·7%). The dehydrated material had m. p. 232° (in bath at 230°) with little darkening. A saturated solution of the hydrate in chloroform at 23° contained approximately 28 mg./ml. The hydrate afforded L-stachydrine picrate, m. p. and mixed m. p. 199—200°, on treatment with picric acid. The hydrate was stable to the air in Khartoum, but deliquescent in the moist air of London. Schulze and Trier (*loc. cit.*) reported that DL-stachydrine hydrate is deliquescent and insoluble in cold chloroform.

The mother-liquor from (A) was extracted ten times with boiling chloroform–ethanol (chloroform, 150 c.c.; ethanol, 5 c.c.). The extracts, filtered hot, were united and evaporated; this gave crude crystals (12·5 g.) which afforded nearly pure L-stachydrine hydrate (5·9 g.) on recrystallisation from cold water.

NATIONAL INSTITUTE FOR MEDICAL RESEARCH,
MILL HILL, LONDON, N.W.7.
WELLCOME CHEMICAL LABORATORIES,
MINISTRY OF HEALTH, KHARTOUM.

[*Received, October 24th*, 1951.]