556. *The Isolation and Identification of (—)-Stachydrine Ethyl Ester Periodide from the Root of* Courbonia virgata.

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The aim of the investigation was to examine the periodides, other than that of tetramethylammonium iodide, precipitated by Wagner's reagent from toxic extracts of the root of *Courbonia virgata*. One such periodide has been isolated in the crystalline state and identified as (—)-stachydrine ethyl ester tri-iodide.

In recent years several references have been made to the incidence of toxic properties amongst the members of the Capparidaceae. Folkers (*J. Amer. Pharm. Assoc.*, 1938, **37**, 689) recorded the presence of a substance with curare-like properties in *Capparis sola*; van der Walt and Steyn (*Onderstepoort J. Vet. Science*, 1943, **18**, 207) demonstrated the toxic properties of *Boscia foetida* Schinz. and *Cadaba juncea*; *Capparis tomentosa* has long had a reputation in the Sudan for being toxic to camels; and Henry (*Brit. J. Pharmacol.*, 1948, **3**, 187) reported the presence in *Courbonia virgata* A. Brongn. of a salt of tetramethylammonium hydroxide (tetramine) in sufficient concentration to render the plant quite highly toxic, the toxic action being a typical curare-like effect in conformity with the known physiological properties of this base. *Courbonia pseudopetalosa* Gilg and Benedict, from Nigeria, has also been shown (Henry, unpublished results) to contain the tetramethylammonium base in about the same concentration as in *C. virgata*. The chemical constituents of *Courbonia virgata* were further investigated by Henry and Grindley (*J. Soc. Chem. Ind.*, 1949, **68**, 9) who reported the presence in the plant of di- and tri-methylamine and the probable presence of other bases, apparently of a quaternary nature, in addition to tetramethylammonium hydroxide.

The method of isolation of tetramine from *C. virgata* employed by Henry (*loc. cit.*) and by Henry and Grindley (*loc. cit.*) consisted in the precipitation of the base as its periodide by addition of a substantial excess of Wagner's reagent (iodine in potassium iodide) to the previously purified ethanol extract of the plant material, decomposition of the periodide by heating it with water, evaporation to dryness, and washing of the residue with ethanol to remove readily soluble iodides and undecomposed periodides (see below), followed by recrystallisation from hot water. Tetramethylammonium periodide, when pure, melts above 100°, and its decomposition to the iodide by heating with water on the water-bath proceeds smoothly, as it is fairly soluble in hot water. The periodide precipitate produced from a purified extract of *C. virgata* plant material contains in addition to tetramethylammonium periodide the periodides of other basic constituents, of much lower melting points and not so readily decomposable by heating with water, together with, apparently, other constituents not removed by basic lead acetate treatment but precipitable by iodine–potassium iodide. It is the further investigation of these residual periodides, and the bases producible from them, which has led to the isolation, as its crystalline periodide, of a quaternary base which has been identified as (—)-stachydrine ethyl ester. It is unlikely that this ester is an artefact produced from the betaine, stachydrine, during the process of isolation. Ethyl esters have been detected in the plant kingdom but infrequently. Molasses are said to contain an ethyl ester which in view of the finding of stachydrine ethyl ester may possibly be betaine ethyl ester.

Experimental.

Isolation of (—)-Stachydrine Ethyl Ester Periodide.—Five kg. of root material of *C. virgata*, air-dried and ground to pass a 30-mesh sieve, were digested for 8 hours on the water-bath, in 1-kg. lots, with rectified spirit (2 l.); the rectified spirit, which was acid in reaction, was filtered off and the residue washed with ethanol, re-extracted in the same way, and filtered. The spirit used for the second extraction of each lot was used for the first extraction of the next lot. The bulk of the spirit was distilled, then the residue was evaporated to a thick liquid by heating on the water-bath for several hours. The residue was diluted to 2·5 l. with distilled water and freed completely from fatty material by a preliminary treatment with molten paraffin wax, followed by several extractions of the cold, filtered solution first with light petroleum and then with chloroform. The solution, still intensely coloured, was then made alkaline with ammonia solution and exhaustively extracted with chloroform, then with ether. These extracts after examination were discarded.

The extracted mother-liquor was treated with neutral lead acetate until the solution became acid, then with basic lead acetate, of which large quantities were required, until no further precipitation took place. The filtrate was then added to four times its volume of ethanol which caused further heavy precipitation, the filtrate from which was evaporated to about 350 c.c. to remove the ethanol and then freed from lead by hydrogen sulphide after extensive dilution. The filtrate, which was still intensely coloured, was evaporated to 350 c.c., ammonium iodide (75 g.) in water (100 c.c.) added to the hot solution, and the solution placed in the refrigerator overnight. This treatment removed the bulk of the tetramine as the iodide, the yield of which was 71 g. The mother-liquor was transferred to a 1·5-l. beaker and heated to 60°, a layer of light petroleum added to sink the periodide, then excess of iodine solution (850 c.c. of 25% iodine in 18% ammonium iodide) added slowly with stirring. The precipitated periodide, which collected as a fluid layer 1 cm. thick at the bottom of the beaker, was covered with a half-inch layer of chloroform, in which it has low solubility, and the supernatant aqueous layer syphoned off as completely as possible. The beaker was then filled with water, and the aqueous layer again syphoned off, this process being repeated several times to effect complete removal of the water-soluble impurities—ammonium iodide, glucose, and fructose (produced by inversion of the sucrose present in the root), potassium salts, acetic acid, etc.—which were present in large quantities. The periodide was collected as completely as possible by the aid of rectified spirit, which was then removed on the water-bath, to give a mobile layer of periodide 1 cm. deep in a 1·5-l. beaker. To make quite certain that all undesirable impurities were removed, the periodide layer was twice treated on the water-bath with water (1 l.), and the aqueous extract discarded. The decomposition of the periodides to the iodides of the bases was now proceeded with, by prolonged heating on the water-bath with much stirring and occasional decanting of the aqueous extract into evaporating dishes and evaporation to low bulk. It was at this stage that the main difficulties began to be encountered as, unlike the decomposition of tetramine periodide which proceeds smoothly, it was found that the iodine clung with great tenacity to the solution, owing to the stability of some of the periodides present.

As decomposition of the periodide proceeded, much of its mobility was lost until, by the time some 30—40% had been decomposed, the residue could be dried to a friable solid, part of which could be dissolved in ethanol leaving a very inactive solid residue which still contained a high proportion of available iodine. The solution containing the bulk of the iodides of the more readily decomposable periodides was treated with purified animal charcoal, filtered, and evaporated almost to dry dryness on the water-bath with much blowing with the fan over the surface, and the residue extracted with absolute ethanol to separate the bulk of the remaining tetramine iodide, which has low solubility in ethanol. When kept, the ethanol solution acquired an intensely dark colour; it was transferred to an evaporating basin and the ethanol evaporated. On treatment of the residue with water a nearly colourless aqueous solution resulted but a substantial globule of fluid periodide (X) was present. After some days, large crystals of a periodide slowly formed in the filtered aqueous solution, which by then had become quite brown. Crystallisation was therefore allowed to proceed for 16 days, by which time a substantial crop had formed. The crystals were collected, washed with ice-cold distilled water and dried (yield G_2 = 1·09 g.). The crystals had only a very slight odour of iodine. The mother-liquor from this crop of crystals was set aside at room temperature, whereupon more crystals, apparently identical with the first crop, soon began to form. After 20 days the crystals were collected as before (yield G_2' = 1·53 g.). Subsequent analysis showed the two crops to be identical. The filtrate from this crop, when kept, produced further crystals of similar appearance to the first two crops, but these proved to be heavily contaminated with a fluid periodide; whilst the fourth crop of periodide which the solution yielded was essentially fluid.

The fluid periodide (X) was dissolved in hot ethanol (*ca.* 100 c.c.) and allowed to cool, substantial crystallisation occurring. The crystals were collected, washed with cold ethanol, and dried to constant weight (yield G_1 = 3·73 g.). The filtrate, after a preliminary concentration, was kept at room temperature until the ethanol had evaporated completely. The periodide residue was at first fluid, but on further storage much crystallisation had occurred. On treatment of the residue with ethanol (5 c.c.) much dissolved, but a substantial crystalline residue remained, which was filtered off, washed with ice-cold ethanol (30 c.c.), then with much ice-cold water, and dried to constant weight (yield G_1' = 1·86 g.). These two crops were shown to be identical with G_2 and G_2'. The total weight of the specimens G (8·2 g.) represents only a small fraction of the original periodide precipitated from the extract of the 5 kg. of root material. At least one other periodide, fluid at the ordinary temperature and readily soluble in ethyl and amyl alcohols, is present and will be further investigated as opportunity occurs.

Identification of the Crystalline Periodide as (—)-*Stachydrine Ethyl Ester Tri-iodide.*—Both specimens of *periodide* G_2 and G_2' crystallised in stout prisms (Found, in G_2: C, 19·6, 19·4; H, 3·5, 3·2; N, 2·7, 2·9; I, 71·4. In G_2': C, 19·8, 19·4; H, 3·3, 3·5; N, 2·7; I, 72·2, 71·9. $C_9H_{18}O_2NI_3$ requires C, 19·5; H, 3·6; N, 2·5; I, 68·6%). The high iodine values, in view of the excellent values for carbon, hydrogen, and nitrogen, are possibly due to salts occluded in the microanalytical method employed.

(—)-*Stachydrine ethyl ester picrate.* The periodide (0·5 g.) was dissolved in 50% ethanol and treated with excess of silver powder. The colourless solution was concentrated to a very small volume (1 c.c.) and treated with excess of saturated aqueous sodium picrate. The *picrate* (0·14 g.) crystallized readily, m. p. 101° (Found: C, 45·9, 44·5, 44·8; H, 4·9, 5·5, 5·0; N, 13·7, 13·9; EtO, 14·3, 20·8, 25·4. $C_9H_{18}O_2N,C_6H_2O_7N_3$ requires C, 45·0; H, 5·0; N, 14·0; EtO, 11·3%). The ethoxy-values were obtained by two independent micro-analysts; the high values are attributed to mobilisation of the *N*-methyl groups, possibly through intermediate methyl ester formation. For (±)-stachydrine ethyl ester picrate Schultze and Trier (*Z. Physiol. Chem.*, 1910, **67**, 87) give m. p. 94—96°.

Optical rotation. An approximate value for this constant was obtained by treating the periodide (1·035 g.) with silver powder in aqueous ethanol, filtering, removing the alcohol, and diluting the residue to 25 c.c. with water; $[\alpha]_D^{20}$ −4·4°, calculated as iodide.

(−)-Stachydrine Ethyl Ester Aurichloride and Platinichloride.—The aqueous iodide solution from the optical-rotation determination was treated with excess of freshly precipitated silver chloride, filtered, and evaporated to dryness. The residual striated mass of crystals was dissolved in water (1 c.c.), and a portion (0·5 c.c.) treated with gold chloride solution. The precipitated oily ***aurichloride*** rapidly crystallized in small needles (0·3 g.), m. p. 61—62°. Schultze and Trier (*loc. cit.*) give m. p. 59—60° for the (±)-salt (Found : C, 21·3; H, 3·5; Au, 38·7. $C_9H_{18}O_2NCl_4Au$ requires C, 21·1; H, 3·5; Au, 38·6%). The remainder of the parent solution of chloride (0·5 c.c.) was treated with chloroplatinic acid solution and gave a platinum salt as clusters of tiny needles, m. p. 195° (decomp.).

Attempted Synthesis of (−)-Stachydrine Ethyl Ester.—L-Proline, $[\alpha]_D^{25}$ −85·5° (1·3 g.), was methylated in methanol solution with sodium hydroxide and excess of methyl iodide (King, *J.*, 1941, 338). The neutral solution was diluted with water, excess of silver chloride was added, and after filtration the solution was evaporated to dryness and the methylated product dissolved in absolute ethanol to remove sodium chloride. At this stage the solution was checked for optical activity and had $[\alpha]_D^{25}$ −24° (calculated on the assumption that the product was stachydrine hydrochloride). Yoshimura and Trier (*Z. physiol. chem.*, 1912, **77**, 292) give $[\alpha]_D^{18}$ −26° for this salt. The ethanolic solution was then treated with 1 pellet of sodium hydroxide and boiled to hydrolyse any methyl ester formed during the methylation process. Aqueous hydrochloric acid was added until the solution was slightly acid, and the solution was then evaporated to dryness and the residue extracted with absolute ethanol to remove sodium chloride. The ethanolic solution was saturated with hydrogen chloride at −5° and then boiled for 3·5 hours. The solution was finally evaporated to dryness under reduced pressure, and the residue dissolved in water (5 c.c.). This solution was now optically inactive. The picrate, aurichloride, and platinichloride were readily obtained in crystalline form and must be regarded as the salts of (±)-stachydrine ethyl ester. A comparison of their m. p.s and mixed m. p. behaviour with the corresponding salts of (−)-stachydrine ethyl ester is shown below.

	(−)-Stachydrine ethyl ester.	Mixture.	(±)-Stachydrine ethyl ester.
Picrate	101—102°	102—103°	103—104°
Gold salt	63—64	59—60	58—60
Platinum salt	195 (decomp.)	195—198	196—198

These salts are extremely difficult to recrystallize without change; a small portion of both picrates on recrystallization separated from water in prisms, both showing m. p. 104—105°. It seems possible that the m. p. of these three salts are the same for the (−)- and (±)-esters.

From the above it is clear that racemization has occurred, probably at the stage when the methanolic solution of (−)-stachydrine or its methyl ester was boiled with alkali. To avoid this, the experiment was repeated but with omission of the alkaline treatment. The ethanolic solution of the neutral methylation product of L-proline was saturated with hydrogen chloride as described above, and an aqueous solution of the ester hydrochloride obtained in the same way. The picrate and gold and platinum salts were obtained crystalline, and a comparison of their m. p.s with those of the natural product from *Courbonia virgata* is shown below.

	(−)-Stachydrine ethyl ester.	Mixture.	Synthetic product from L-proline.
Picrate	101—102°	96—97°	96—97°
Gold salt	63—64	65—68	70—73
Platinum salt	195 (decomp.)	199 (decomp.)	204

The lack of agreement shown in this table is pronounced, and the conclusion is drawn that the synthetic product prepared in this way must contain some methyl ester from the original methylation process. In this connection, the m. p. 70—73° observed above for the gold salt may be significant since Jahns (*Ber.*, 1896, **29**, 2067) gives m. p. 85° for (±)-stachydrine methyl ester aurichloride.

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