107. *The Presence of* cis- *and* trans-3-*Hydroxystachydrine in the Fruit of* Courbonia virgata.

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During the preliminary investigation of the basic constituents of the fruit of *Courbonia virgata* dextro-rotatory forms of both *trans*- and *cis*-3-hydroxystachydrine have been isolated. These compounds form approximately 10% of the dry weight of the husks of the fruit. One of the compounds was also isolated from the kernels, but neither compound has so far been found in the root of the plant.

THE mature fruit of *Courbonia virgata* A. Brongn. contains a single kernel, measuring approximately 15 × 10 × 7 mm. and enclosed in a husk consisting of hard brown epicarp and cream-coloured endocarp. The kernels represent about 80% of the weight of the whole dry fruit. They are free from soluble carbohydrates and contain lower proportions of tetramethylammonium salts and inorganic constituents than do the roots (cf. Henry and Grindley, *J. Soc. Chem. Ind.*, 1949, **68**, 9). These factors would be expected to facilitate isolation of any other basic constituents which might be present. The fruit has a pronounced " fishy " odour which is retained for long periods in a closed vessel. The kernels used in this work were not quite free from tenaciously adhering endocarp.

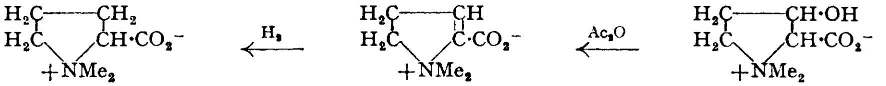
Extraction of the husk material with ethanol has now given two crystalline, water-soluble, isomeric compounds, m.p. *ca.* 250° and 209—210°, respectively, which we shall distinguish by the suffixes -*a* and -*b* respectively. Similar treatment of the kernels has yielded the former, whereas root material under the same conditions gave only tetramethylammonium nitrate. The presence of L-stachydrine ethyl ester salts in the root has been already established (Henry and King, *J.*, 1950, 2866).

A preliminary examination of the isomer-*a* was made by Dr. H. King, F.R.S. It dissolves readily in water to a neutral, dextrorotatory, non-conducting solution not precipitated by picric acid, sodium picrate, mercuric chloride, iodine in potassium iodide, Tanret's reagent, or by chloroauric or chloroplatinic acid; precipitates are given by phosphotungstic acid and by ammonium reineckate. The hydrochloride and the picrate crystallize readily from ethanol, and the analyses of these and of the original compound-*a* indicate the formula $C_7H_{13}O_3N$. When heated alone, the compound-*a* liberates carbon dioxide and a vapour which gives a weak pyrrole pine-splinter test; when heated with strong alkali, it liberates a volatile base.

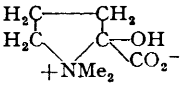
The compound-*b*, of m.p. 209—210°, also affords a neutral, non-conducting aqueous solution, more strongly dextrorotatory than that of its isomer. A crystalline hydrochloride, but no picrate, could be obtained from it.

When either isomer is boiled for a short time with acetic anhydride, an optically inactive anhydro-compound, $C_7H_{11}O_2N$, is formed in good yield. Hydrogenation of this in an acidic medium affords DL-stachydrine (I) as the main product. The isomers $C_7H_{13}O_3N$ are therefore hydroxystachydrines and the anhydro-compound is the methyl betaine of a 1-methylpyrroline-2-carboxylic acid. The position of the double bond in the pyrroline was shown by oxidation with cold dilute permanganate; β-dimethylaminopropionic acid was identified as a major oxidation product by the analysis and melting point of its hydrochloride. Hence the anhydro-compound has the structure (II).

The structures (III) and (IV) now come into consideration for the isomers-*a* and -*b*. The latter (IV), however, should be tautomerically unstable, since it is the quaternary salt of a carbinolamine; moreover, both isomers cannot have this structure as they are not enantiomorphs. If it is admitted that migration of a double bond may take place in the formation of the anhydro-betaine, structures (V) and (VI) have to be considered. However, structure (VI) is open to the same objection of tautomeric instability, and structure (V) belongs to the known betaines, turicine and betonicine, neither of which from their properties could be identical or enantiomorphous with our compounds-*a* and -*b*. The latter can therefore be confidently identified as stereoisomerides of 3-hydroxystachydrine (III); provisionally, isomer-*b* might be regarded as a *cis*-form because of its greater solubility and slighter tendency to form crystalline salts. The facile dehydration of the 3-hydroxystachydrines is consistent with their formulation as β-hydroxy-acids.



(I) (II) (III)



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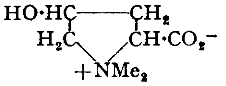

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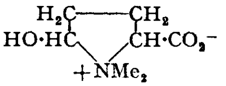
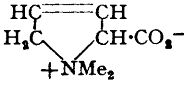


(VI)

The 3-hydroxystachydrines-*a* and -*b* must differ in configuration at only one carbon atom, but it is not known whether this is $C_{(2)}$ or $C_{(3)}$ (betonicine and turicine differ at $C_{(2)}$). An attempt to produce $C_{(2)}$-epimers from 3-hydroxystachydrine-*b* by boiling aqueous barium hydroxide, a reagent which racemizes L-stachydrine, gave only the anhydro-compound.

In the hydrogenation product of the anhydro-compound, DL-stachydrine is accompanied by a substance, isolated as the picrate, having the composition of a dimethylaminovaleric acid; and when the hydrogenation is conducted in the absence of added acid this becomes the main product and is apparently accompanied by a little dimethylammonium valerate. Carbon–nitrogen bond hydrogenolysis could hardly take place under these conditions with stachydrine (I) or with the Δ^2-pyrroline (II), but would be normal with the Δ^3-pyrroline (VII) which is an allylammonium ion twice over. Since the structure (VII) is excluded for the anhydro-compound by the oxidation experiment, it seems that a rearrangement (II ⟶ VII) takes place under the influence of the platinum catalyst. In support of this



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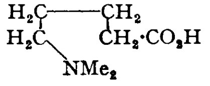
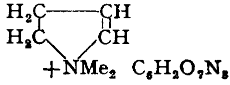


(VIII)



(IX)

view is the fact that an unusually long " lag period " has always preceded hydrogenation. The hydrogenolysis product is probably δ-dimethylaminovaleric acid (VIII) rather than the α-isomer.

When 3-hydroxystachydrine-*a* is boiled for some hours with acetic anhydride, carbon dioxide is slowly evolved and from the darkened solution the methopicrate of a methylpyrroline can be prepared. This is presumably 1-methyl-Δ^2-pyrroline methopicrate (IX).

We intend, when the opportunity arises, to study the stereochemistry of the 3-hydroxystachydrines and to attempt their synthesis.

EXPERIMENTAL

*Isolation of 3-Hydroxystachydrines-*a *and -*b *from the Husks.*—The husk material (280 g.), air-dried and ground, was extracted at room temperature with 95% ethanol, first by percolation and then by digestion overnight. The combined extracts (1·8 l.) were allowed to evaporate and the residue was taken up in water (110 c.c.). After acidification with sulphuric acid (3 drops of 2N) and extraction with light petroleum to remove fat, the solution was concentrated, first with gentle heating and later over sulphuric acid in a desiccator. The resulting crystallization was facilitated by frequently breaking the surface crust. Four successive crops (6·1, 3·4, 6·5, 4·8 g.), melting at about 250° (decomp.) and consisting essentially of the isomer-*a*, were obtained in this way, small amounts of cold ethanol being used to wash, and when necessary to collect, the crystals. Further extraction of the husk material with boiling 95% ethanol (500 c.c.) for 3½ hours yielded further material (5·1 g.) of good quality by a similar procedure. The crude material was recrystallized by dissolution in water and concentration as before. After two such treatments, final purification was effected by pouring a concentrated aqueous solution into cold ethanol. *3-Hydroxystachydrine-*a separated in well-formed colourless prisms, melting with effervescence at about 250° (variable with rate of heating) and having $[\alpha]_D^{30}$ + 10·0° (*c*, 29 in water) (Found: C, 52·9, 52·7; H, 8·1, 8·2; N, 8·9, 9·0. $C_7H_{13}O_3N$ requires C, 52·8; H, 8·2; N, 8·8%). The yellow *picrate* separated readily from ethanol; it had m. p. 160° (Found : C, 40·2; H, 4·1; N, 14·1, 14·6. $C_7H_{13}O_3N,C_6H_3O_7N_3$ requires C, 40·2; H, 4·1; N, 14·4%). The *hydrochloride* crystallized from ethanol in needles, m. p. 196—197° (decomp.), not hygroscopic (Found : N, 7·5. $C_7H_{13}O_3N,HCl$ requires N, 7·2%).

The mother-liquor from the final crop of crude 3-hydroxystachydrine-*a* was allowed to dry over sulphuric acid. After 10 days the viscous, crystal-bearing matrix was filtered without addition of ethanol and sucked as free as possible from mother-liquor. The crude crystals were dissolved in water (10 c.c.) and the solution, after treatment with charcoal (0·3 g.) and filtration, was allowed to evaporate at room temperature to small bulk. The residue was cooled in a refrigerator and stirred with ethanol (5 c.c.); the resultant large crystals were collected, washed with cold ethanol (10 c.c.), and air-dried (2·46 g.). This product had m. p. 210—212° (decomp.) after drying at 110°. It was dissolved in water and the filtered solution was concentrated to 5 c.c., poured into cold ethanol (25 c.c.), and left in the refrigerator. Next day the colourless prisms of *3-hydroxystachydrine-*b *monohydrate* (1·87 g.), m.p. 209—210° (decomp.), $[\alpha]_D^{22}$ + 53° (*c*, 2·5 in water), were collected, washed with ethanol and then acetone, and air-dried (Found : N, 8·0; loss at 100—110°, 10·4, 10·45. $C_7H_{13}O_3N,H_2O$ requires N, 7·9; H_2O, 10·2%. Found, on dried material : C, 53·3; H, 8·0; N, 9·4%). The *hydrochloride* crystallized from dry ethanol in prisms, m.p. 201—202° (decomp.) (Found : N, 7·0%). The mother-liquors from the crude 3-hydroxystachydrine-*b* contained 19 g. of solids which will be further investigated.

*Isolation of 3-Hydroxystachydrine-*a *from the Kernels.*—The ground material was extracted (Soxhlet) with light petroleum, and the residue (810 g.) digested twice with ethanol at room temperature. The combined ethanol extracts (2 l.) were allowed to evaporate. An aqueous solution of the residue, after exhaustive extraction with light petroleum and with ether–chloroform, was allowed to evaporate, finally over calcium chloride. Ethanol (10 c.c.) was stirred into the viscous, partly crystalline mass before filtration, which was very slow. The residue, after being washed with ethanol, was dissolved in water and the solution evaporated to small bulk after treatment with charcoal. The well-formed crystals of 3-hydroxystachydrine-*a* (4·0 g.) were collected and washed with alcohol, and had m.p. 248—250° (decomp.). The mother-liquor contained 30 g. of solids which are being further studied.

Examination of the Root Material.—Dried and ground root (1 kg.), previously extracted with light petroleum and with chloroform, was digested twice with ethanol (1400 c.c) at room temperature. The extract was allowed to evaporate and the residue was dissolved in water. The filtered solution, after extraction with light petroleum and then with ether–chloroform, was examined for extractable bases by being made alkaline and extracted with ether–chloroform; it was then acidified and concentrated, finally over sulphuric acid, to a viscous mass containing crystals. The latter were collected by means of ethanol, dissolved in water (10 c.c.), treated with charcoal, and recovered by evaporation to small bulk and addition of ethanol (3 c.c.). The product (1·46 g.) has been identified as tetramethylammonium nitrate. There is no evidence at present for the presence of either of the 3-hydroxystachydrines in the root of *Courbonia virgata.*

Action of Acetic Anhydride on the 3-Hydroxystachydrines.—3-Hydroxystachydrine-*a* (1·568 g.) was boiled with acetic anhydride (8 c.c.) until dissolution was complete (a few minutes). The solution was concentrated at low pressure and water was added; after the remaining acetic anhydride had reacted, the mixture was evaporated once or twice at low pressure with addition of ethanol. The crystalline residue was taken up in a little ethanol; pyridine (1 c.c.) was added, and then ethyl acetate to incipient turbidity. The crystalline, nearly pure 1 : 1-*dimethyl*-Δ^2-*pyrrolinium-2-carboxylic betaine* (II) (1·088 g.) was collected next day. Recrystallization from ethanol–ethyl acetate gave well-formed prisms, m.p. about 235° (decomp.; variable). The substance was hygroscopic; an aqueous solution was neutral and showed no detectable optical activity (Found : C, 59·6; H, 8·1; N, 10·4. $C_7H_{11}O_2N$ requires C, 59·6; H, 7·8; N, 9·9%). The *picrate* separated from ethanol in stout, four-sided yellow prisms, m.p. 174—175° (Found : C, 42·0; H, 3·6; N, 14·6. $C_7H_{11}O_2N, C_6H_3O_7N_3$ requires C, 42·2; H, 3·8; N, 15·1%). When pyridine was omitted in the initial crystallization of the pyrroline-betaine, a crystalline mixture of it and its acetate separated.

3-Hydroxystachydrine-*b* on similar treatment with acetic anhydride gave the anhydro-compound in substantially the same yield. The picrate had m.p. 174—175° alone or mixed with the previous specimen (Found : C, 41·8; H, 3·8; N, 15·3%). The anhydro-compound was isolated in good yield as the picrate after 3-hydroxystachydrine-*b* (500 mg.) had been boiled with barium hydroxide (1 g.) in water (20 c.c.) for 3 hours. The reaction mixture then showed no optical activity.

3-Hydroxystachydrine-*a* (159 mg.) was heated (bath, 150°) with acetic anhydride (1 c.c.) in a slow current of nitrogen. Carbon dioxide was detected in the emergent gas by passing it through aqueous barium hydroxide. After about 3 hours the acetic anhydride was removed at low pressure and ethanolic picric acid was added to the residue. One crystallization from methanol and two from ethanol (much tar had to be separated) gave deep yellow long prismatic needles (30 mg.), m.p. 284° (decomp.; in bath at 280°) of 1 : 1-*dimethyl*-Δ^2-*pyrrolinium picrate* (IX) (Found : C, 43·8; H, 4·6; N, 17·2. $C_6H_{12}N,C_6H_2O_7N_3$ requires C, 44·2; H, 4·3; N, 17·2%).

Hydrogenation of the Anhydro-compound.—The anhydro-compound (300 mg.) in hydrochloric acid (5 c.c.; N) with platinum oxide (18 mg.) was shaken with hydrogen at atmospheric temperature and pressure. After reduction of the catalyst further absorption of hydrogen did not begin for some minutes. Thereafter the uptake was rapid until 1·1 mols. had reacted; the catalyst then coagulated and absorption ceased (this sequence of events has been observed repeatedly in this reduction). The solution was filtered and evaporated at low pressure. Aqueous sodium picrate (12 c.c.; 4·3%) was added to the residue; the mixture was evaporated at low pressure and the solid collected with the help of a little water. The dried product was boiled with ethyl acetate, an orange residue remaining undissolved. This appeared to be a stachydrine picrate containing less than one equivalent of picric acid: on recrystallization from ethanol four-sided orange leaflets were obtained, having m.p. 194—195° depressed to 180° on admixture with DL-stachydrine picrate (Found : C, 45·2; H, 4·7; N, 14·0%). However, when this substance was recrystallized from ethanol containing picric acid, yellow leaflets, m.p. and mixed m.p. 195°, of DL-stachydrine picrate were obtained (Found : C, 41·7; H, 4·3; N, 14·6. Calc. for $C_7H_{13}O_2N,C_6H_3O_7N_3$: C, 41·9; H, 4·3; N, 15·1%).

The ethyl acetate extract after concentration deposited prisms which were recrystallized from alcohol to give yellow acicular prisms, m.p. 153—154°, of (?) δ-*dimethylaminovaleric acid picrate* (Found : C, 41·4; H, 5·1; N, 14·5. $C_7H_{15}O_2N,C_6H_3O_7N_3$ requires C, 41·7; H, 4·8; N, 15·0%).

When the anhydro-compound was hydrogenated in the absence of hydrochloric acid the uptake of hydrogen was greater and the picrate, m.p. 153—154°, was the only product isolated. The solution smelled of valeric acid when acidified and of a volatile amine, presumably dimethylamine, when made alkaline.

Oxidation of the Anhydro-compound.—The anhydro-compound (0·5 g.) in a little water was cooled to 0° and treated slowly with 4% aqueous potassium permanganate. After addition of 50 c.c. the initially rapid reaction became slower; the process was interrupted after addition of a further 10 c.c. The filtered solution was acidified with hydrochloric acid and evaporated at low pressure. The residue was extracted with cold ethanol. Evaporation of the ethanol left a crystalline residue which was recrystallized from ethanol and formed leaflets (115 mg.), m.p. about 175°. Three more recrystallizations raised the m.p. to 187—188° (Clarke, Gillespie, and Weisshaus, *J. Amer. Chem. Soc.*, 1933, **55**, 4571, give m.p. 188—191° for β-dimethylaminopropionic acid hydrochloride) (Found: C, 39·1; H, 7·4; N, 9·1. Calc. for $C_5H_{11}O_2N,HCl$: C, 39·1; H, 7·8; N, 9·1%). The m.p. was strongly depressed by admixture with dimethylaminoacetic acid hydrochloride.

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