which was extracted with EtOAc. The residue after EtOAc was extracted with $CHCl_3$–MeOH (2:1) and chromatographed on activated charcoal, eluted by EtOH–H_2O (1:99), giving L-(+)-bornesitol (0·01% of roots, $[\alpha]_D$, m.p., m.m.p., IR and TLC).

Plant. Amsonia elliptica Roem et Schult. Cultivated at medicinal botanic garden of our university. *Uses.* None. *Previous work.* Cyclitols in sister species, *A. angustifolia* Michx and *A. Tabernaemontana* Walt.[1]

Leaves and stems. Extracted with MeOH. The extraction procedure was the same as described above. L-(+)-bornesitol (0·006% of leaves and stems, $[\alpha]_D$, m.p., m.m.p., IR and TLC).

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ALKALOIDS FROM *COURBONIA GLAUCA* RHIZOME

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Key Word Index—*Courbonia glauca*; Capparaceae; tetramethylammonium; (±)-3-hydroxy-1,1-dimethylpyrrolidinium; (±)-stachydrine.

Plant. Courbonia glauca[1–3] (synonyms: *C. edulis* Gilg. & Bened.,[1–3] *C. camporum* Gilg. & Bened.,[1,3] *Maerua edulis* Gilg. & Bened.[2]). *Source.* Zambesi Valley, Rhodesia. *Uses.* Native fish poison.

Previous work: Quarternary ammonium compounds have been reported in *C. camporum* Gilg. & Bened.,[4] tetramethylammonium,[5,6] di- and tri-methylamine,[6] (−)-stachydrine ethyl ester[7] and *cis* and *trans* 3-hydroxystachydrine[8] in *C. virgata* A. Brongn. (synonyms: *M. pseudopetalosa* Gilg. & Bened., *C. pseudopetalosa* Gilg. & Bened.[3]). L-Stachydrine[9] was isolated from the fruit of *Capparis tomentosa* Lam., a member of the same family.

Present work. Since betaines and pure quarternary ammonium compounds are not extractable into water-immiscible solvents, indirect methods for their isolation must be employed; for this reason the procedures used are described at some length. Some evidence for the presence of saponins was found, but no attempt to isolate and identify them was made.

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[2] KILLICK, D. J. B. (1970) in *Flora S. Africa*, Vol. 13, p. 165, Govt. Printer, Pretoria.
[3] ELFFERS, J., GRAHAM, R. A. and DEWOLF, G. P. (1964) *Flora of Tropical East Africa—The Capparidaceae*, p. 43, Crown Agents, London.
[4] WATT, J. M. and BREYER-BRANDWIJK, M. G. (1962) In *Medicinal and Poisonous Plants of Southern and Eastern Africa*, p. 163, Livingstone, London.
[5] HENRY, A. J. (1948) *Br. J. Pharmacol.* **3**, 187.
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[7] HENRY, A. J. and KING, H. (1950) *J. Chem. Soc.* 2866.
[8] HENRY, A. J. and CORNFORTH, J. W. (1952) *J. Chem. Soc.* 597.
[9] CORNFORTH, J. W. and HENRY, A. J. (1952) *J. Chem. Soc.* 601.

Rhizome, oven-dried at 55°, was milled and 2·0 kg Soxhlet-extracted with cyclohexane, $CHCl_3$ and MeOH in sequence. The MeOH extract was concentrated to 200 ml, diluted to 500 ml with H_2O and filtered to provide solution *A*. $CHCl_3$ extracts of solution *A* before and basification with KOH failed to yield basic material extractable into dil. HCl. Boiling a basified aliquot of solution *A* liberated no volatile amines. *Courbonia glauca* rhizome therefore contains neither volatile bases nor alkaloids extractable from neutral or alkaline aqueous medium by water-immiscible solvents.

Solution *A* was slightly acidified, basic lead acetate solution added until neutral, and neutral 10% lead acetate added until no further precipitation occurred (240 ml). The filtered solution was nearly neutralized with NH_3, re-filtered, re-treated with neutral lead acetate (40 ml of 20%) and re-filtered. The filtrate was concentrated to 200 ml, stood overnight and $PbCl_2$ removed by filtration. The filtrate was diluted to 600 ml, the Pb precipitated with excess H_2S, and excess H_2S removed at 60° (solution *B*, 450 ml). Solution *B* contained 11% w/v sucrose (TLC and titration. This represents 2·4% of dry wt extracted) but no reducing sugars (negative Barfoed and Fehling tests).

Solution *B* gave a heavy precipitate with Wagner's reagent (K periodide solution). 200 ml was columned on Amerlite IR-120 (Na) (700 ml) (Col. 1). Elution with H_2O gave a (betaine + sugar) fraction, from which the sugars were separated by a second column, Amberlite IR-120 (H) (200 ml), eluting with H_2O. The sugar-containing solution, acid in reaction, was decolourized with charcoal. The *laevo* rotation was not inconsistent with the presence of D-glucose and D-fructose in equimolar amounts, and it yielded a single osazone, d.p. 210°.

Quaternary salts. Following removal of sugars and betaines, elution of Col. 1 with 4% aq. NaCl gave the quaternary salt fraction. After concentration, addition of a moderate excess of K periodide (20% I_2 in 40% aq. KI) gave a massive precipitate of crystalline organic periodide (X), which was filtered and reduced to iodide by digestion with H_2O at 100° and finally shaking in H_2O with Ag powder. The crude iodide (11·0 g), recrystallized from H_2O, gave tetramethylammonium iodide, d.p. 230°[5] (10·4 g, tetramethylammonium representing 0·45% of dry wt extracted), identified by comparison with a synthetic sample. (Found: C, 24·0; H, 6·3; N, 6·5; I, 63·5. Calc. for $C_4H_{12}NI$: C, 23·9; H, 6·0; N, 7·0; I, 63·2%). The aurichloride was prepared (d.p. 334°) and analysed satisfactorily. The picrate gave a d.p. 327–9° (315°).[5] No other alkaloid was present in *X* in appreciable quantity.

On addition of a large excess of strong KI_3 solution to the mother liquor from X, and standing, a mat of slender needles formed. On separation and reduction with Ag powder this yielded crude iodide (0·49 g). After conversion to the chloride and further purification, treatment with 5% aq. chloroauric acid afforded, after recrystallization from H_2O, (±)-3-hydroxy-1,1-dimethylpyrrolidinium aurichloride, d.p. 243–4° (260°)[10] (264 mg, 3-hydroxy-1,1-dimethylpyrrolidinium representing 0·008% of dry wt extracted). (Found: C, 15·9; H, 3·1; N, 3·0; Au, 43·2; Cl, 31·2. Calc. for $C_6H_{14}NOAuCl_4$: C, 15·8; H, 3·1; N, 3·1; Au, 43·3; Cl, 31·2%). The identity was established spectroscopically: IR (KBr) ν 3500 cm^{-1}; NMR (d_6-DMSO) τ 7·5–8·4 *m* [C(4)H_2]; 6·95 *s* [2 × NMe]; 6·50 *t* [C(5)H_2]; 6·34 *d* [C(2)H_2]; 5·55 *m* [C(3)H]; 4·90 broad *s* [OH].[11] The picrate, prepared via the hydroxide, showed d.p. 250–2° and analysed satisfactorily. This compound has not previously been reported as occurring naturally.

Betaines. Separation of Solution *B* (200 ml) into (betaines + sugars) and quaternary salts was repeated, using Col. 1, After further purification of the (betaine + sugar) fraction with

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[11] MANDAVA, N. and FODER, G. (1970) *Annalen* **741**, 167.

$Pb(OAc)_2$–H_2S, and adjustment of the final vol. to 350 ml, the betaines were precipitated as a globular mass of periodides (50 g) with hydrogen periodide (120 ml 16% I_2 in 14% HI plus 32 g I_2 in 35 ml conc. HI). This was transformed to a solution of iodides as above, which was shaken with an aqueous suspension of excess silver chloride and filtered. Picric acid (5·3 g) was added to the total filtrate (220 ml) at the boiling point, resulting in the recovery, after purification of combined fractions by recrystallization from water, of (±)-stachydrine picrate, m.p. 198–199° (199–200°)[9] (7·2 g, stachydrine representing 0·47% of dry wt extracted). (Found: C, 41·77; H, 4·18; N, 15·21. Calc. for $C_{13}H_{16}N_4O_9$: C, 41·94; H, 4·30; N, 15·05%). The identity was established by comparison with a synthetic sample and spectroscopically: IR (KBr) ν 1725 cm^{-1}; NMR (4:1 $CDCl_3/d_6$-DMSO) τ 7·2–8·1 m [C(3)H_2 + C(4)H_2]; 6·82 *s* [NMe]; 6·59 *s* [NMe]; 6·21 *t* [C(5)H_2]; 5·41 *t* [C(2)H]; 1·55 *s* [OH]; 1·39 [2 × aromatic CH].[12] The (±) stachydrine isolated represents only some 25% of the total betaines present in the rhizome.

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STEROL CONTENT OF *SPINACIA OLERACEA*

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Key Word Index—*Spinacia oleracea*; Chenopodiaceae; spinach; sterols; α-spinasterol; stigmastenol; stigmastanol.

Hart[1] reported the isolation of sterols from the spinach, *Spinacia oleraceae* L. fat which was subsequently identified as α-spinasterol.[2]

Processing fresh leaves of Egyptian spinach in the usual manner gave 0·003% unsaponifiable matter (on wet wt basis). This material was resolved by chromatography on an alumina column. Elution with *n*-hexane followed by C_6H_6 containing 15% $CHCl_3$ gave a crystalline material which exhibited a positive Liebermann–Burchardt test and a yellow colour reaction with the tetranitromethane reagent. The product was thought to be pure as judged from the constant m.p. (147–149°) and optical rotation, $[\alpha]_D$ −45°. The acetate derivative, m.p. 169°, $[\alpha]_D$ −8°, and the benzoate derivative, m.p. 187°, $[\alpha]_D$ −10·7° were also prepared. The acetylated material was inspected by TLC[3] and found to be homogenous,

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[3] COPIUS-PEEREBOOM, J. W. (1964) *Z. Anal. Chem.* **205,** 325.