Hyenanchin and other constituents of Hyenache globosa / by Thomas Anderson Henry.

CLXXXII.—*Hyenanchin and other Constituents of* Hyenanche globosa.

By Thomas Anderson Henry.

Hyenanche globosa, Lamb (*Toxicodendron capense*, Thun), is the sole member of a genus of the natural order Euphorbiaceæ, and in distribution is confined to South Africa. The name of the plant is said to have originated from the use of the seeds in South Africa as a poison for wild animals, especially hyenas.

In 1858 Henkel obtained, by fractionation of a concentrated alcoholic extract of the fruits, a syrupy preparation, which proved to be highly toxic (*Arch. Pharm.*, 1858, **144**, 16). Engelhardt subsequently prepared the toxic constituent, which he named hyenanchin, in a crystalline condition, but, beyond stating that it was neither an alkaloid nor a glucoside, made no attempt to characterise it (*Arb. pharm. Inst. Dorp.*, 1892, **8**, 5). Shortly afterwards, E. Merck (*Merck's Ann. Report*, 1895, 123) pointed out that if the observation, made by Henkel and by Engelhardt, that hyenanchin exerts a strychnine-like action on the brain, but is without action on the spinal cord, is trustworthy, the substance might be of some therapeutic value as a substitute for strychnine in cases where cerebral action alone is required.

For a supply of the plant, the author is indebted to Mr. I. B. Pole Evans, Chief of the Division of Botany, Department of Agriculture, Pretoria.

Experimental.

The material used included stems (1 kilo.), leaves (2 kilos.), and fruits (4 kilos.). Each of these parts of the plant was extracted separately with chloroform in a continuous, hot percolation apparatus, yielding, in the case of the stem and leaves, a hard, dark green wax, and in that of the fruits a neutral, dark yellow oil, which was not examined in detail. Extraction was continued with hot 95 per cent. alcohol, and the liquid concentrated to a thin syrup, which was poured into five volumes of water, causing the separation of sparingly soluble tannin containing a small amount of a yellow colouring matter. The filtrate, after purification with lead acetate in the usual manner, was concentrated under reduced pressure to a viscid syrup, which was then extracted repeatedly with an equal volume of ethyl acetate until it was free from bitterness and no longer toxic. The brown, sticky residue left on distilling off the ethyl acetate was dissolved in three times

its weight of water and set aside, when, after some weeks, a bitter, poisonous, crystalline material separated. Mere traces of this were obtained from the leaves and stems, and only 6 grams from the fruits (0·15 per cent.), but this does not represent all that is present, as the mother liquor was still toxic and intensely bitter, and in the case of the fruits a further 1·7 grams (making a total yield of 0·19 per cent.) were obtained by repeating the treatment with ethyl acetate.

This crude material could only be recrystallised by adding it to fifty times its weight of boiling water and filtering rapidly, when, on cooling, there separated a crop of slender, colourless needles. Slight concentration of the filtrate gave a further crop of the same substance, and reduction of the mother liquor from this crop to half its original volume led to the separation of a second substance, crystallising in short, colourless, hexagonal prisms, of which more was obtained by further concentration. This second substance forms about two-thirds of the original crystalline material, and it is proposed to apply to it Engelhardt's name, hyenanchin; as the substance crystallising in needles is an isomeride of this, it may appropriately be called iso*hyenanchin*.

Hyenanchin.

This substance, isolated as described above, and purified by repeated recrystallisation from boiling water, has no melting point, but when heated becomes yellow at 200°, then darkens, and finally decomposes sharply, with effervescence, at 234°. It dissolves in water to the extent of 1·18 per cent. at 15°, is more soluble in boiling water, and sparingly so in alcohol, butyl alcohol, ethyl acetate, or acetone. It has $[\alpha]_D^{15} + 14·7°$ in water (Found: C = 58·2, 58·3, 58·05, 57·9; H = 6·03, 6·01, 6·4, 6·3. Loss at 110° in a vacuum, nil. $C_{15}H_{18}O_7$ requires C = 58·06; H = 5·8 per cent.).

Hyenanchin contains no nitrogen, reduces Fehling's solution on boiling, and silver nitrate solution on gently warming or on keeping in the cold, decolorises permanganate immediately, and gives an amorphous, yellow precipitate with bromine water. Boiling dilute hydrochloric acid converts it into an amorphous, brown substance, which gives a characteristic, orange-coloured, amorphous precipitate with phenylhydrazine in the cold. No sugar is produced. On warming with alkalis, hyenanchin furnishes a distillate containing a minute quantity of a substance, which gives the iodoform reaction, reduces Fehling's solution, and furnishes a semicarbazone, crystallising in short, colourless needles, and decomposing at 200°, which may be acetolsemicarbazone, $C_4H_9O_2N_3$ (decomp. 195—200°) (Found: N = 31·12. Calc.: N = 32·06 per

cent.). A specimen of acetolsemicarbazone, prepared under similar conditions, and without recrystallisation, gave N = 30·8 per cent., and a mixture of the two decomposed at 198°. No derivatives of hyenanchin with hydroxylamine, phenylhydrazine, or semicarbazide could be obtained.

Action of Baryta.—Although hyenanchin is not acid to indicators, it neutralises alkalis, but its titration presents difficulty, owing to the ease with which it becomes coloured and decomposes in presence of alkalis. If a solution of hyenanchin to which excess of baryta has been added is warmed or kept for several days, it becomes brown and turbid, the latter due to the gradual separation of barium carbonate. By dissolving it in *N*/5-baryta and at once titrating with *N*/5-sulphuric acid in the presence of phenolphthalein, a fairly satisfactory end-point is obtained (Found: $Ba(OH)_2$ required for neutralisation, 55·2, 55·05. Calc. for two CO_2H groups, 55·3 per cent.).

On evaporating in a vacuum the filtrate from the barium sulphate formed, barium carbonate is gradually deposited, and the filtrate from this yields, on complete evaporation in a vacuum, a nearly colourless varnish, which appears to consist chiefly of a barium salt of a monocarboxylic acid, since it yields on addition of excess of *N*/5-sulphuric acid 29·8 per cent. of its weight of barium sulphate ($C_{28}H_{42}O_{14}Ba$ requires $BaSO_4 = 31·5$ per cent.). The corresponding *acid*, obtained by adding the calculated quantity of *N*/5-sulphuric acid to an aqueous solution of this salt and evaporating the filtrate to dryness in a vacuum, is a colourless varnish, which is readily soluble in water, and reduces Fehling's solution on boiling and ammoniacal silver nitrate in the cold. The acid does not regenerate hyenanchin on drying, even at 150° in a vacuum.

These results indicate that hyenanchin is probably a dilactone, convertible by the carefully regulated action of weak alkali into the corresponding dihydroxydicarboxylic acid, $C_{15}H_{22}O_9$, which is unstable and readily loses one carboxyl group.

Action of Acetic Anhydride.—When hyenanchin is heated with acetic anhydride at 100°, most of it crystallises out unchanged on cooling, but if a drop of pyridine be added and the heating continued, it is converted into a soft, sticky resin, from which with great difficulty there can be separated, by repeated crystallisation from dilute alcohol, a small yield of crystalline material consisting of at least three substances: (*a*) cream-coloured needles, softening at 136° and finally melting and decomposing at 169°; (*b*) colourless needles, m. p. 126°; (*c*) colourless, short needles, m. p. 104°. Only a few centigrams of each of these substances were obtained,

so that they could not be satisfactorily purified for examination, but it is probable that only the second is a true acetyl derivative of hyenanchin (Found: C=56·7, 57·05; H=5·7. $C_{15}H_{17}O_7Ac$ and $C_{15}H_{16}O_7Ac_2$ require C=57·9; H=5·6 per cent.).

iso*Hyenanchin.*

iso*Hyenanchin* crystallises from boiling water in long, slender needles with a silky lustre, has no melting point, but becomes brown at 245° and decomposes sharply, with effervescence, at 299°. It dissolves in water to the extent of 0·26 per cent. at 15°, is somewhat more soluble in boiling water, and less so in alcohol or ethyl acetate. $[\alpha]_D^{15}$ −61·3° in water (Found: C=58·08, 58·25; H=5·97, 6·16. $C_{15}H_{18}O_7$ requires C=58·06; H=5·80 per cent.). It reduces Fehling's solution on boiling and ammoniacal silver nitrate in the cold.

Action of Baryta.—*iso*Hyenanchin dissolves immediately in excess of *N*/5-baryta solution, forming a solution, which only becomes slightly yellow after remaining several days at 0°, and under these conditions combines with sufficient baryta to neutralise one carboxyl group (Found, on immediate titration, 29·8; after three days at 0°, 29·2. Calc. for one CO_2H group, 27·7 per cent.). On again adding excess of *N*/5-baryta solution and boiling gently for a few minutes and titrating back, baryta equivalent to a second carboxyl group is found to have been absorbed (Found: 25·9. Calc.: 27·7 per cent. Total for two CO_2H groups=55·4. Calc.: 55·3 per cent.). A second estimation, made by heating *iso*hyenanchin with excess of *N*/5-baryta solution for one hour at 100° and titrating back, gave 56·2 per cent. The filtrate from these estimations, on concentration, behaved like the similar preparation from hyenanchin (p. 1621), and gave as a final residue an amorphous barium salt, yielding 29·1 per cent. of barium sulphate on precipitation with *N*/5-sulphuric acid.

Physiological Action of Hyenanchin and iso*Hyenanchin.*

The author is greatly indebted to Dr. J. Trevan, of the Wellcome Physiological Research Laboratories, who kindly undertook the examination of a series of preparations of *Hyenanche*, and finally of the pure substances isolated. Dr. Trevan reports that hyenanchin has a physiological action almost identical in kind with that of picrotoxin, but is much weaker. *iso*Hyenanchin, on the contrary, is not toxic in such doses as can be injected intravenously.

Relationship of Hyenanchin and iso*Hyenanchin to other Non-nitrogenous Convulsant Poisons.*

The reactions described above and the physiological action of hyenanchin indicate that the latter belongs to the group of convulsant, non-nitrogenous poisons, of which picrotoxinin, $C_{15}H_{16}O_6$ (m. p. 206·5°, $[\alpha]_D$ +4°40′ in alcohol), coryamirtin, $C_{15}H_{18}O_5$ (m. p. 225°, dextrorotatory), and tutin, $C_{17}H_{20}O_7$ (m. p. 208—209°, $[\alpha]_D$ +9·25° in alcohol), are the only well-defined members known. Associated with picrotoxinin in the molecular compound, picrotoxin, $C_{30}H_{34}O_{13}$, is the substance, picrotin, $C_{15}H_{18}O_7$ (m. p. 245—246°, $[\alpha]_D$ −55·2° in water), which is not toxic. Picrotoxinin and picrotin are both dilactones, and are now believed to contain, respectively, one and two hydroxyl groups, the function of the sixth and seventh oxygen atoms, respectively, being still unknown (Horrmann, *Annalen*, 1916, **411**, 273). Both these substances yield small quantities of acetone on distillation with alkali. Picrotin is isomeric with hyenanchin and *iso*hyenanchin, and in many ways is very similar to the latter. The two have therefore been carefully compared, and found not to be identical, the chief differences being that picrotin has a definite melting point and is completely converted into the corresponding dicarboxylic acid by baryta in the cold, whilst *iso*hyenanchin has no definite melting point, and is converted into the corresponding dicarboxylic acid by baryta in two well-defined stages. A mixture of both substances begins to sinter at 225°, which is well below the melting and decomposing points of the two components. A number of substances of the formula $C_{15}H_{18}O_7$ have also been prepared from picrotoxinin and picrotin (compare Horrmann, *loc. cit.*), namely, picrotin–lactone (338°, decomp.), picrotoxic acid (m. p. 230—231°, $[\alpha]_D$ +81·7°, crystallises with $2H_2O$), α-picrotoxinic acid (m. p. 209°, $[\alpha]_D$ −48°), and β-picrotoxinic acid (m. p. 235°, $[\alpha]_D$ −48°). None of these closely resembles either hyenanchin or *iso*hyenanchin.

Subsidiary Constituents of Hyenanche globosa.

Examination of the Wax. Isolation of a New Phytosterol and a New Wax Alcohol.

The dark green wax from the leaves and stems was mixed with an equal weight of animal charcoal, and extracted with boiling ethyl acetate, yielding a greenish-yellow solution. This, on cooling, deposited a mixture of two substances, which were separated by heating the solution to boiling and adding enough ethyl acetate

to keep both substances dissolved at 35°. On keeping, the liquid then deposited gelatinous granules, which filtered with difficulty, and, on drying in the air, formed a pale brownish-green, horny mass. This was purified by distillation in a vacuum and recrystallisation from ethyl acetate, when it formed colourless masses of minute needles melting at 82—83° (corr.). A number of solid alcohols melting near this temperature are known, but all of them differ slightly in composition and most of them in crystalline form from hyenanche alcohol, and of those tried, ceryl, myricyl, and wheat* alcohols, all depressed the melting point. It is readily soluble in chloroform, boiling ethyl acetate, or alcohol, and sparingly so in ether, does not combine with bromine, and appears to be a new saturated *alcohol* of the formula $C_{24}H_{49}\cdot OH$ (Found, in substance dried at 60° in a vacuum: C = 81·02, 81·27, 81·19†; H = 13·5, 13·55, 13·89.† $C_{24}H_{50}O$ requires C = 81·3; H = 14·1 per cent.).

When boiled for several hours with acetic anhydride in presence of pyridine, it yields an *acetyl* derivative, which is readily hydrolysed on recrystallisation from most solvents, but separates from acetic anhydride in soft masses of colourless needles melting at 75° (corr.) (Found: C = 78·8; H = 13·0. $C_{26}H_{52}O_2$ requires C = 78·7; H = 13·1 per cent.).

Carnaubyl alcohol, which also has this formula, crystallises in leaflets (m. p. 68—69°), and is quite distinct from the alcohol of *H. globosa*. The other known alcohols of this formula are either liquid or of much lower melting point than *Hyenanche* alcohol.

The filtrate from the alcohol on concentration deposited a second substance, which, after repeated crystallisation from boiling ethyl acetate, forms long, lustrous needles melting at 265° (corr.), is readily soluble in chloroform or boiling ethyl acetate, and sparingly so in alcohol, even on boiling (Found: C = 83·66, 83·95; H = 11·35, 11·66. $C_{28}H_{46}O$ requires C = 84·4; H = 11·55 per cent.). $[\alpha]_D^{15}$ −22·4° in chloroform.

It gives a typical phytosterol reaction with sulphuric acid in the presence of acetic anhydride, and furnishes a *monoacetyl* derivative, crystallising from hot ethyl acetate in small, spheroidal masses of colourless needles melting at 244° (corr.) (Found: C = 81·12; H = 10·96. $C_{30}H_{48}O_2$ requires C = 81·8; H = 10·9 per cent.).

This substance appears to be a new phytosterol belonging to the series represented by the general formula $C_nH_{2n-10}O$, of which at least eight are now known, beginning with alcornol, $C_{22}H_{34}O$

* For a specimen of this alcohol the author is indebted to Mrs. M. T. Ellis (*Biochem. J.*, 1918, **12**, 160). † Regenerated from acetyl derivative.

(Hartwich and Dünnenberger, *Arch. Pharm.*, 1900, **238**, 348), and terminating with amyrin, $C_{30}H_{50}O$ (Windaus and Welsch, *ibid.*, 1908, **246**, 506). The new phytosterol is exceptional in this series in being lævorotatory and in giving an acetyl derivative melting at a lower temperature than the parent substance, but in all other respects it resembles other members of the series.

Isolation of a New Yellow Colouring Matter.

The colouring matter was obtained in small amount (total, 2·3 grams crude, 1·0 gram pure, from all three sources) by extracting the sparingly soluble tannin (p. 1619) with boiling dry ether, and was recrystallised from alcohol, from which it separated on slow evaporation in microscopic, yellow needles, which became brown at 200°, and finally melted and decomposed at 270—280°. It is moderately soluble in alcohol, sparingly so in ether, and insoluble in chloroform. The solution in alcohol gives a brownish-black precipitate with ferric chloride (Found: C=62·43, 62·1,* 62·1*; H=4·4, 4·4,* 4·25.* Loss in a vacuum at 100°, 10·08. $C_{15}H_{12}O_6$ requires C=62·5; H=4·16. $C_{15}H_{12}O_6,2H_2O$ requires loss 11·1 per cent.).

The colouring matter furnished an *acetyl* derivative, crystallising from hot alcohol in masses of cream-coloured needles melting at 234—236° (decomp.; corr.) (Found: C=63·4; H=4·0. Loss at 60° in a vacuum, nil). On regeneration with hydrochloric acid in the presence of hot acetic acid, the acetyl derivative yielded 71·3 per cent. of colouring matter identical with the original substance. Sulphuric acid could not be used for this purpose, as it appeared to convert the colouring matter into a soluble sulphonic acid (compare A. G. Perkin, T., 1899, **75**, 448). These results indicate that the acetyl derivative should be represented by the formula $C_{15}H_7O_5Ac_3$ (requires C=63·6; H=4·04; colouring matter, 72·7 per cent.), which is a triacetyl derivative of the parent substance, less one molecule of water. The reactions of the colouring matter indicate that it belongs to the flavone group, in which the loss of a molecule of water on acetylation does not appear to have been recorded previously, except doubtfully in the case of morin (A. G. Perkin, *loc. cit.*). Unfortunately, the small amount of material available precluded further investigation of this and other points.

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* Regenerated from acetyl derivative.

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