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SHORT REPORTS

THE OCCURRENCE OF 4-HYDROXYISOLEUCINE IN STEROIDAL SAPOGENIN-YIELDING PLANTS

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Key Word Index—*Dioscorea deltoidea*; Dioscoreaceae; *Balanites aegyptiaca*; Balanitaceae; *Trigonella foenum-graecum*; Leguminosae; *Solanum laciniatum*; Solanaceae; 4-hydroxyisoleucine; steroidal sapogenin.

Plants. Dioscorea deltoidea tuber purchased in 1967 from Seth Panchhi Ram & Co., Kuth Grower, Manali, Kulu Hills, India. *Balanites aegyptiaca* seed purchased as fruits from Nigeria in 1966 from United Africa Co., Liverpool. *Trigonella foenum-graecum* commercial seed from Morocco, purchased in 1970 from Evans Gray & Hood Ltd., 110 Cannon Street, London, E.C.4; freed from weed seed. *Solanum laciniatum* fruit grown at Bath in 1973 from seed supplied by Searle de Mexico, S.A. de C.V.

Previous work. 4-hydroxyisoleucine from seeds of *Trigonella foenumgraecum* [1]. Fenugreekine, a new steroidal sapogenin-peptide ester of *Trigonella foenum-graecum* [2].

Present work [3]. Dry powdered plant material was extracted with 20% EtOH, solvent removed, residue dissolved in H_2O and purified on cation exchange resin. The amino acid fraction was displaced with N NH_3, conc., residue dissolved in H_2O and (2S,3R,4R)-4-hydroxyisoleucine detected by Co-TLC using silica gel and (a) phenol–H_2O (3:1), R_f 0·37 and (b) BuOH–HOAc–H_2O (4:1:1), R_f 0·43 (15 cm). The amount of free 4-hydroxyisoleucine was determined by densitometric TLC using a Joyce Loebl Chromoscan on the transmittance mode 0·06% (*Dioscorea*), 0·03% (*Balanites*), 0·11% (*Solanum*) and 0·74% (*Trigonella*), m.f.b. (2S,3R,4R)-4-Hydroxyisoleucine 200 mg/kg in 4 ml H_2O *per os* in male dutch rabbit: negative hypoglycaemic activity.

Comment. This appears to be the first record of the detection and quantitative determination of free (2S,3R,4R)-4-hydroxyisoleucine in higher plants, other than fenugreek (age of plant material may have reduced yield). All are steroidal sapogenin (or their N-analogue)-yielding plants of four distinct families (Dioscoreaceae, Balanitaceae, Leguminosae and Solanaceae) suggesting the co-occurrence of such saponins with 4-hydroxyisoleucine. This also suggests that the sapogenin-peptide ester, fenugreekine [2], which on hydrolysis affords a mixture of three isomeric 4-hydroxyisoleucine lactones [2], is the first example of a class of compounds that will be of common occurrence in steroidal saponin containing plants, including those investigated above and others, such as species of *Digitalis*.

Fenugreekine is reported to have pharmacological activities which include hypoglycaemic and cardiatonic properties [2]. Some amino acids are known to stimulate insulin secretion [4]. In a preliminary test the free (2S,3R,4R)-4-hydroxyisoleucine showed no hypoglycaemic activity.

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THE DEMONSTRATION OF GALACTOSAMINE IN A HIGHER PLANT: *CANNABIS SATIVA**

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Key Word Index—*Cannabis sativa*; Cannabinaceae; marijuana; glycoprotein; amino sugar; galactosamine.

Plant and source. Cannabis sativa was grown from South African seeds in the Botanical Garden of University of Oslo. Identification of the plants was carried out by Prof A. Nordal, Department of Pharmacognosy, Insti-

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* This investigation was carried out in collaboration with the United Nations Narcotics Laboratory in Geneva, as part of the U.N. Cannabis research programme established by resolution 8 (XIV) of the Commission on Narcotic Drugs.

tute of Pharmacy, University of Oslo. *Previous work.* [1,2].

Plant part examined. Dried leaves and stems of *Cannabis sativa* were milled and extracted with light petrol. After drying, the residual material was extracted with H_2O at 40° under stirring for 2 hr followed by dialysis against H_2O. The non-dialysable material was purified by ion exchange chromatography on DEAE-cellulose and subsequent gel giltration on Sepharose 4B. The product consisted of polysaccharide with protein attached.

The component sugars were analysed by GLC after methanolysis and trimethylsilylation of the resulting methyl glycosides [3]. The trimethylsilyl ethers were separated on a column (200 × 0·3 cm) of SE 52 on Varaport 30, using a N_2 gas flow of 33 ml/min and a temperature program with start at 140° and an increase of 2°/min for 10 min, followed by an increase of 4°/min. Under these conditions the derivatized *N*-acetylgalactosamine and *N*-acetylglucosamine appeared at 197° and 200°, respectively. Quantitative GLC with mannitol as the internal standard resulted in a content of 6·7% *N*-acetylglucosamine and 3·1% *N*-acetylgalactosamine. The presence of the two amino sugars was confirmed by automatic amino acid analysis on a BioCal BC 200 Analyzer following acid hydrolysis with 6 N HCl at 110° for 20 hr. By this method a content of 5·3% *N*-acetylglucosamine and 1·9% N-acetylgalactosamine was found. The lower values obtained by the latter procedure are explained by partial degradation of hexosamine, known to occur under strong conditions of hydrolysis [4].

The purified biopolymer (0·2 g) was hydrolysed with 4 N HCl at 100° for 6 hr. After evaporation of HCl the hexosamines and amino acids were adsorbed to a column (2·2 × 6 cm) of AG 50 × 8 (H^+) resin. Neutral sugars were removed by washing with water followed by eluting the column with 2 N HCl (40 ml). The acid eluate was concentrated to dryness, and the residue subjected to TLC on Si gel in the solvent system (v/v) *n*-PrOH–NH_3 25% (13:7), and to PC in the solvent systems (v/v) EtOAc–pyridine–HOAc–H_2O (5:5:1:3), and *n*-BuOH–pyridine–1 N HCl (5:3:2) using as locating reagents: (a) Aniline oxalate or $AgNO_3$–NaOH, (b) ninhydrin, and (c) periodic acid–benzidine [5]. In all three solvent systems spots were obtained having the mobility and giving the same colour reactions upon spraying as the authentic hydrochlorides of glucosamine and galactosamine. Mannosamine hydrochloride, also run as a reference, was clearly separated from the two other hexosamines.

It is concluded that galactosamine is present as an integral part of a carbohydrate–protein polymer in *Cannabis sativa*. In the quantitative analyses the results are given as *N*-acetylhexosamine since these sugars almost always occur as *N*-acetyl derivatives in nature. Whereas glucosamine has been found to occur in tissues of a number of higher plants [6], the presence of galactosamine, although reported [7,8] has never been established conclusively.

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SUCROSE OCTAACETATE FROM *CLEMATIS*

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Key Word Index—*Clematis chinensis*; *C. apiifolia*; Ranunculaceae; bitter material; sucrose octa-acetate.

We wish to report the isolation of sucrose octa-acetate as a natural material from *Clematis* spp. This is the first occasion that a disaccharide peracetate has been found as a natural product.

From the fraction extracted with ether of dried Radix Clematidis (roots of *Clematis japonica*), which has been used as a diuretic or rheumatic, needles mp 84–86°, having a bitter taste were obtained in a yield of 0·15%. On the basis of IR, NMR and mass spectral evidence [1, 2] and the elementary analysis, a formula $C_{12}H_{22}O_{11}$-$(C_2H_2O)_8$ was given to this compound, that of an octaacetyl disaccharide. Finally, it was identified as sucrose octa-acetate by comparison of its TLC, IR, NMR and mass spectra with those of the authentic sample prepared by the known method [3] and by its failure to depress the m.p. of an admixture with the synthetic sample. The octa-acetate was also found in freshly collected roots of *Clematis chinensis* Osbeck and *C. apiifolia* DC. and is regarded as a new type of naturally occurring bitter principle.

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