*Acknowledgements*—We are indebted to Dr. G. Englert and W. Meister, F. Hoffmann-La Roche, Basel, for recording and interpretation of the NMR and MS. This work was supported by Deutsche Forschungsgemeinschaft (SFB 46, Universität Freiburg).

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*Phytochemistry*, Vol. 21, No. 1, pp. 239–240, 1982.
Printed in Great Britain.

0031–9422/82/010239–02 $03.00/0


# A TRIGLUCOSIDE OF HECOGENIN FROM FRUITS OF *AGAVE CANTALA*

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(*Received* 3 *March* 1981)

**Key Word Index**—*Agave cantala*; Agavaceae; saponin; hecogenin triglucoside.

**Abstract**—The fruits of *Agave cantala* contain a steroidal saponin which is a glycoside of hecogenin with three molecules of glucose. Its structure has been established as α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 3)hecogenin.

A review of the literature showed that while leaves of different *Agave* species contain a number of steroidal saponins [1] no work has been reported on the fruits of these plants.

The blackish fruits of *Agave cantala* were therefore collected from Srinagar–Garhwal, and dried and powdered. The defatted powder was extracted with ethanol which was concentrated to give a cream-coloured syrupy mass, which was purified in the usual manner [2]. The light cream-coloured powder obtained on TLC showed the presence of one major component, which was finally purified by column chromatography.

The purified substance (**1**) had mp 240–243°, $[\alpha]_D$ +95.5° (*c*, 1% MeOH), and its IR spectra showed bands at 860, 900, 920 and 980 $cm^{-1}$ indicating that ring F is closed and there is no sugar attached to C-26. On acid hydrolysis (2 N $H_2SO_4$) it yielded a genin (44.8%), identified as hecogenin by mmp and co-chromatography with an authentic sample. The hydrolysate after neutralization was deionized with ion-exchange resins [Amberlite IRA 400 and IR 120 ($H^+$)]. The solution thus obtained was concentrated to a syrupy mass which by PC, GLC (silyl derivative) [3] and HPLC showed only the presence of glucose. The molar ratio of aglycone:glucose was 1:3 based on genin weight.

In order to determine the sequence and mode of linkage of sugars **1** was completely methylated and this was confirmed by IR spectra. The permethylated saponin was subjected to methanolysis and the resulting methyl glycosides of the methylated sugars thus obtained were identified by GLC as 2,3,4,6-tetra-*O*-methyl-α-D-glucose (1 mol), and 2,3,6-tri-*O*-methyl-α-D-glucose (2 mol). On enzymic hydrolysis with β-glucosidase, no sugar was liberated indicating that the glucose molecules are α-linked. On treatment with $NaIO_4$ no free sugar was liberated, suggesting that all three glucose molecules are linked in such a manner that they are all oxidized by periodate. The molecular rotation calculations of the saponins based on Klyne's rule [4] also support the α-linkage of the sugars. The observed $(M)_D$ value, +874.78, is in reasonable agreement with the calculated $(M)_D$ value, +882.38.

Thus on the basis of above results **1** is identified as α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 4)α-D-glucopyranosyl(1 → 3)hecogenin.

## EXPERIMENTAL

All specific rotations are equilibrium values. Whatman filter paper No. 1 was used for PC: for sugars the solvents were *n*-BuOH–pyridine–$H_2O$ (6:4:3) or EtOAc–pyridine–$H_2O$ (22:10:1) and sprayed with *p*-anisidine HCl or alkaline $AgNO_3$. TLC of **1** was carried out on Si gel in *n*-BuOH–HOAc–$H_2O$ (4:1:5) or $CHCl_3$–MeOH–$H_2O$ (13:7:2). The sprays were 5% $H_2SO_4$ or cinnamaldehyde reagent. GC was carried out using 8% OS-138 on Chromosorb-W (NAW) column with $N_2$ and FID. HPLC was carried out using column—μ Bondapak/carbodydrate, solvent system acetonitrile–$H_2O$ (4:1).

*Isolation and purification of* **1**. Dried and powdered fruits of *Agave cantala* collected from plants growing in Srinagar–Garhwal were defatted (petrol 40–60°), followed by

exhaustive extraction with 95% EtOH. The extract was worked up as described in the text [2] and monitored by TLC. Final purification was by CC on Si gel using $CHCl_3$–MeOH–$H_2O$ (13:7:2) as eluant. Fraction A gave **1** as a light cream-coloured compound, mp 240–243°, $[\alpha]_D$ +95.5° (*c*, 1% MeOH), IR $\nu_{max}$ 860, 900, 920, 980 $cm^{-1}$.

*Isolation of genin and sugars.* Acid hydrolysis with 2 N $H_2SO_4$ was carried out in the usual manner (see text). The genin was crystallized from MeOH, mp 263–264° and identified as hecogenin. The deionized supernatant was concd and examined by PC and gave glucose only. This was confirmed by GLC using pyridine, hexamethyldisilazine and trimethylchlorosilane for silylation. The silyl derivative of the sugar was identified as glucose by GLC ($R_t$ and peak enhancement method). Further confirmation was by HPLC.

*Methylation and methanolysis of the saponin.* The saponin was methylated in the usual manner in DMSO with MeI and NaH The fully methylated product was isolated and showed no absorption in the 3-$\mu$m region in IR, indicating the absence of free OH groups. The methylated product was subjected to methanolysis with 3% methanolic HCl in a sealed tube at 70° for 5 hr. The soln was neutralized ($AgCO_3$) and filtered.

*Identification of methylated sugars.* The methyl glycosides of methylated sugars obtained were identified by GLC on 8% OS-138 on Chromosorb-W (NAW) column as 2,3,4,6-tetra-*O*-methyl-α-D-glucose and 2,3,6-tri-*O*-methyl-α-D-glucose by comparison with authentic samples. From the GLC trace the molar ratio was determined as 1:2 by the peak area method.

Periodate treatment for 48 hr with 0.05 M $NaIO_4$ soln was followed by extracting with *n*-BuOH. The *n*-BuOH extract after evapn and hydrolysis with 2 N $H_2SO_4$ showed no sugars on PC.

*Enzyme hydrolysis.* Treatment of **1** in NaOAc buffer (pH5) with β-glucosidase at 37° for 50 hr did not release any sugar.

*Acknowledgement*—Thanks are due to the Council of Scientific and Industrial Research, New Delhi, for the award of Senior Research Fellowship to one of us (D.C.J.).

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*Phytochemistry*, Vol. 21, No. 1, pp. 240–241, 1982
Printed in Great Britain.

0031-9422/82/010240-02 $03.00/0


# A COUMARIN FROM *LIMONIA ACIDISSIMA*

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(*Received* 8 *April* 1981)

**Key Word Index**—*Limonia acidissima*; Rutaceae; root-bark; dihydrosuberenol; coumarin; 2 H-1-benzopyran-2-one, 6-[3-hydroxy-3-methyl 1-butyl]-7-methoxy.

**Abstract** Dihydrosuberenol, a new coumarin, was isolated from the methanol extract of the defatted root-bark of *Limonia acidissima*. Its structure has been established through spectral investigations and partial synthesis.

In continuation of our work on *Limonia acidissima* L. [1], we now report the presence of a new coumarin from the MeOH extract of the defatted root-bark. According to *Index Kewensis* [2] the valid name of this species is *L. acidissima* and not *L. crenulata* or *Hesperthrusa crenulata* [3]. The plant material for investigation was collected locally and a voucher specimen (386 and 388) has been preserved at the University Herbarium in the Botany Department.

The extract was chromatographed on a Si gel column and eluted with solvent mixtures of increasing polarity. A pale yellow solid, obtained from the $C_6H_6$–$CHCl_3$ (1:1) eluate was rechromatographed and on monitoring by TLC it has been possible to obtain a TLC and GC pure colourless crystalline material (**1**), mp 105°. It analysed for $C_{15}H_{18}O_4$ ($M^+$, 262) and gave the usual colour reactions for coumarins exhibiting $\lambda_{max}^{MeOH}$ at 320, 296 (*sh*), 253 and 224 nm typical of 7-alkoxy coumarins [4, 5]. The IR spectrum in KBr showed peaks at 3500 (–OH), 1700 (⟩C=O), 1595, 1540 (aromatic), 1370, 1355 (*gem* dimethyls), 1260 (⟩C=C–O–C asymmetrical stretching), 1120 (⟩C–O stretching). The $^1H$ NMR data (Table 1) clearly indicate the oxygen functions as methoxyl and hydroxyl. The typical coumarin doublets appeared at δ 6.30 and 7.68 ($J$ = 10 Hz), accompanied by the 6-proton methyl singlet at δ 1.38. The commanding feature in identifying a $-CH_2-CH_2-$(AA'XX') system was apparent from the signals which occurred as multiplets possessing a symmetry about the mid-point of the pattern and the spectrum due to the AA' nuclei was the exact mirror image of that of XX'. The occurrence of two singlets in the aromatic region obviously denotes the presence of two hydrogens at C-5 and C-8 and thereby provides space for the 3-hydroxy-3-methyl-1-butyl side-chain and the