Phytochemistry, Vol 24, No 7, pp 1617–1618, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

HYPOLAETIN 8-GLUCOSIDE FROM *SIDERITIS LEUCANTHA*

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(*Revised received* 22 *November* 1984)

Key Word Index—*Sideritis leucantha*; Labiatae; 5,7,8,3′,4′-pentahydroxy-flavone 8-β-D-glucoside; nuclear methylation.

Abstract—A new naturally occurring hypolaetin 8-β-D-glucoside has been isolated and identified from *Sideritis leucantha*. The natural compound suffered nuclear methylation during derivatization.

Aerial parts of *Sideritis* species are used in Spanish folk medicine for treating acute and chronic inflammation and a flavonoid has been isolated as being responsible for this activity [1]. In continuation of our work on the flavonoids of *Sideritis* [2–4], we have now isolated and identified from *Sideritis leucantha* Cav. a new naturally occurring hypolaetin 8-β-D-glucoside and permethylation with methyl iodide [5] yielded a major C-6 methylated product.

The UV values of the glucoside in the presence of classical reagents [6–8] indicated the existence of free hydroxyls at the 5-, 7-, 3′- and 4′-positions as well as the presence of one supplementary substituent on the A-ring which, by comparison with data obtained for onopordin [8], may be located at C-8. This was confirmed after enzymic hydrolysis where, besides glucose, the aglucone so obtained showed the same chromatographic and UV characteristics as hypolaetin [8, 9]. Moreover, the electron impact mass spectrum was also as expected. The $[M]^+$ at *m/z* 302 revealed five hydroxyl groups on the flavone nucleus, three on ring A and two on ring B, as deduced from the retro-Diels–Alder fragmentation ($[B_1]^+$, *m/z* 134; $[B_2]^+$ *m/z* 137 and $[A_1]^+$ *m/z* 168). Finally, isosinensetin was obtained by diazomethane methylation of the aglycone, verified by TLC with an authentic sample.

Permethylation by means of methyl iodide [5] is a usual derivatization technique in flavonoid glycoside mass spectral identification [10–12] and it is not usual to obtain *C*-methylated compounds as a major product. Nevertheless, it has been reported [13, 14] that nuclear methylation by methyl iodide in 5,7-dihydroxyflavones, occurs at C-6. So we have obtained a C-6 methylated compound during the permethylation process [5]. As suggested, the electron impact mass spectrum of the permethylated derivative, in which we found an $[M]^+$ (*m/z* 590), a $[A+H]^+$ (*m/z* 372) and the retro-Diels–Alder fragment $[A_1-CO]^+$ (*m/z* 181), which were 14 mass units higher than expected, was in accord with the *c*-methyl introduced. This nuclear methylation was corroborated by the electron impact mass spectrum of the per deuteromethylated derivative, in which the peaks $[M]^+$ (*m/z* 617), $[A+H]^+$ (*m/z* 387) and $[A_1-CO]^+$ (*m/z* 190) were 17 mass units higher than expected. Similar *C*-methylation has been described previously for flavanones [10]. The molecular ion at *m/z* 590 showed that the natural glycoside was a monoglucoside.

EXPERIMENTAL

Plant material and extraction This was carried out as described previously [4]

Isolation of the glycoside. From the *n*-BuOH extract, the glycoside was isolated by PC on Whatman No. 1 with 30% HOAc (R_f 0.25) and with BAW (4:1:5, upper phase) (R_f 0.55), and by prep. TLC on polyamide DC-6 (Macherey–Nagel) with H_2O–*n*-BuOH–HOAc–EtOAc (70:10:10:10) (R_f 0.33). UV λ_{max}^{MeOH} nm: 349, 290 sh, 270, 258; $\lambda_{max}^{MeOH+NaOMe}$ nm: 411, 333 sh, 272; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 432, 332 sh, 302 sh, 272; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 395 sh, 356, 298, 277, 262 sh; $\lambda_{max}^{MeOH+NaOAc}$ nm: 394, 323, 278; $+NaOAc+H_3BO_3$, 382, 277 sh, 265.

Permethylation. This was achieved by standard procedures [5]. Permethylated derivative TLC silica gel EtOAc (R_f 0.50), $CHCl_3$–EtOAc–Me_2CO (5:4:1) (R_f 0.74). EIMS (probe) 70 eV, *m/z* (rel. int.): permethylated: 590 $[M]^+$ (3), 373 $[A+2H]^+$ (13), 372 $[A+H]^+$ (58), 357 $[A+H-Me]^+$ (20), 328 $[A+H-COMe]^+$ (3), 218 $[T_1]^+$ (13), 188 (3), 187 $[T_2]^+$ (24), 181 $[A_1-CO]^+$ (10), 167 (3), 165 $[B_2]^+$ (5), 155 $[T_3]^+$ (21), 111 (98), 101 (100), perdeuteromethylated: 617 $[M]^+$ (4), 388 $[A+2H]^+$ (23), 387 $[A+H]^+$ (100), 386 $[A]^+$ (9), 371 (10), 369 $[A-CD_3]^+$ (36), 230 $[T_1]^+$ (13), 196 $[T_2]^+$ (19), 190 $[A_1-CO]^+$ (8), 171 $[B_2]^+$ (2), 161 $[T_3]^+$ (141)

Enzyme hydrolysis This was achieved by β-glucosidase (Sigma) 1 hr, 30°, 0.1 M acetate buffer pH 4.6 The aglycone obtained showed the same chromatographic, UV and MS values as hypolaetin

Acidic hydrolysis. This was carried out with aq 2 M HCl, 2 hr, 80°, yielding hypolaetin and 6-hydroxyluteolin identified by MS and UV procedures

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Phytochemistry, Vol 24, No 7, pp 1618–1619, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

2,3-DIMETHYLNONACOSANE AND TROPANE ALKALOIDS FROM *HYOSCYAMUS ALBUS*

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(*Revised received* 21 *August* 1984)

Key Word Index—*Hyoscyamus albus*; Solanaceae; hyoscyamine, hyoscine; 2,3-dimethylnonacosane.

Abstract—In addition to hyoscine and hyoscyamine, a new compound isolated from the leaves and stems of *Hyoscyamus albus* has been characterized as 2,3-dimethylnonacosane by spectral studies.

INTRODUCTION

Hyoscyamus albus is known to contain tropane alkaloids [1, 2] which have mydriatic, anti-spasmodic and anti-cholinergic properties. This plant has also been reported to possess chlorogenic acid [3]. A re-investigation of this plant has shown it to contain a new branched hydrocarbon and a total crude alkaloid content of *ca* 0.24%, of which *ca* 40% consists of hyoscyamine, *ca* 30% of hyoscine and *ca* 15% of two unidentified bases.

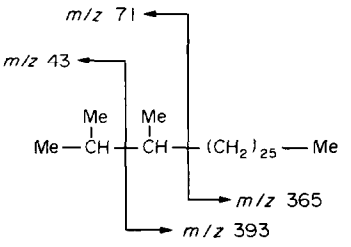
RESULTS AND DISCUSSION

Silica gel column chromatography of the *n*-hexane extract of the plant yielded a crystalline isolate. The isolate, mp 62°, had IR absorption bands at 2960, 2920, 2850, 1465, 720 (long chain) and 1375, 1360, 1150 cm^{-1} (isopropyl group) [4]. An $[M]^+$ ion at *m/z* 436 in the mass spectrum of the compound suggested the molecular formula $C_{31}H_{64}$. The formation of an ion at *m/z* 421 $[M-Me]^+$ indicated the compound to be of branched chain nature carrying a methyl group as substituent. The intensity of the C_nH_{2n+1} peaks after a maximum at $n = 4$, steadily declined up to $n = 25$, followed by a strong peak at $n = 26$ (*m/z* 365). From the intense peak at $n = 26$ $[M-71]^+$, it followed that one methyl group is attached at C-3 [5]. The other significant ions at *m/z* 393 and 43 indicated the presence of a second methyl group at C-2 which is also a part of the isopropyl group. These fragmentations are depicted in structure **1**. The ^{1}H NMR spectrum of the compound integrated for 64 protons. One of the terminal methyl groups was seen as a triplet ($J = 4$ Hz) at δ0.84 and the two methyls of the isopropyl function appeared as a doublet ($J = 6$ Hz) at δ0.80. The methyl group at C-3 was also present as a doublet ($J = 5$ Hz) at δ0.76. These data led us to establish the structure of this compound as 2,3-dimethylnonacosane (**1**).

The structure of compound **1** is in full agreement with the data now available. It has not been found previously in nature. It is interesting to note that the Solanaceae is a good source of branched hydrocarbons. Earlier they were reported from *Solanum torvum* [6] and *Duboisia myoporoides* [7]. Fractions obtained after **1** contained large amounts of oil, which was not worked up further.

EXPERIMENTAL

Mp is uncorr. The IR spectrum was recorded in KBr and the 90 MHz ^{1}H NMR spectrum in $CDCl_3$ with TMS as internal ref. TLC was carried out on silica gel G and the spots were visualized by exposure to I_2 vapour or Dragendorff spray. Plant material



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