Phytochemistry, Vol. 21, No. 2, pp. 472–473, 1982.
Printed in Great Britain.

0031-9422/82/020472-02$03.00/0
Pergamon Press Ltd.

1β-HYDROXYNEOTIGOGENIN, A SAPOGENIN FROM *SOLANUM POLYADENIUM* LEAVES

S. OSMAN, S. L. SINDEN,* P. M. GREGORY,† A. BAKER and K. SEIDEN

Eastern Regional Research Center, Philadelphia, PA 19118, U.S.A.; *Beltsville Agricultural Research Center, Beltsville, MD 20705, U.S.A.; †Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853, U.S.A.

(*Revised received* 22 *June* 1981)

Key Word Index—*Solanum polyadenium*; Solanaceae; sapogenin; 1β-hydroxyneotigogenin.

Abstract—A new sapogenin has been isolated from leaves of *Solanum polyadenium* P.I. 161728, a clone that is highly resistant to Colorado potato beetle and potato leaf hopper. The structure of this compound has been established as 1β-hydroxyneotigogenin, 5α-spirostan-1β,3β-diol.

INTRODUCTION

The species *Solanum polyadenium* is highly resistant to both Colorado potato beetle [1] and potato leaf hopper [2]. Although the mechanism of resistance has not been elucidated, it has been shown that the leaf tissue of this species contains high levels of the glycoalkaloid, tomatine, and an unidentified saponin [Gregory, P., Sinden, S.L., Osman, S. F. and Chessin, D. A., unpublished observations]. Hydrolysis of this saponin yielded a sapogenin of unknown structure. We now report the characterization of the sapogenin and partial characterization of the parent saponin.

RESULTS AND DISCUSSION

The sapogenin was isolated from acid hydrolysates of crude leaf extracts (95% EtOH extracts) and purified by CC and prep. TLC followed by recrystallization; mp 215°; $[\alpha]_D^{25}$ −71° ($CHCl_3$; *c* 0.0670).

High resolution MS showed a molecular ion at *m/z* 432.3240 corresponding to $C_{27}H_{44}O_4$. The base peak at *m/z* 139 was strongly indicative of a spirostan skeleton unsubstituted in the E and F ring [3]. The compound had no significant UV absorption above 210 nm and reacted with acetic anhydride–pyridine to form a diacetate (M^+ at *m/z* 516) suggestive of a hydroxyspirostanol. The mass spectrum of the TMS derivative had a molecular ion at *m/z* 576 (2OHs) and a base peak at *m/z* 217, which is consistent with a 1,3-diol (TMSO-C–$\overset{+}{C}$–C–OTMS). Mild oxidation [4] of the sapogenin resulted in the formation of a diketone (M^+ at *m/z* 428) which showed weak UV absorption (λ_{max} 246, 253, 255, 264) indicative of enol–keto conjugation. ^{13}C NMR assignments (Table 1) were based on data in the literature for cholestanols [5], dehydroxysteroids [6], and sapogenins [7]. The only structure consistent with all the spectroscopic data is 1β-hydroxyneotigogenin. The 1,3-substitution was further confirmed by dehydration of the sapogenin diene according to the method of Hutchins [8]. The product from this reaction (M^+ at *m/z* 396) had λ_{max} 262, which corresponds to a homoannular conjugated diene. On the basis of the ^{13}C NMR spectrum [5, 6] the OHs are diequatorial.

Table 1. ^{13}C NMR spectral data of polygenin

C	δ	δ(literature values [5–7])	C	δ	δ(literature values [5–7])
1	77.9	79.0	15	32.0	31.8
2	42.3	42.5	16	80.8	80.7
3	67.9	69.0†	17	62.2	62.2
4	38.0	38.2	18	16.4	16.5
5	42.3	44.9	19	6.8	6.7
6	28.4	28.6	20	41.5	41.6
7	32.0	32.3	21	14.3	14.5
8	35.6	35.2	22	109.8	109.5
9	54.9	54.4	23	27.1	27.1
10	42.3	42.5	24	25.8	25.8
11	24.3	24.7	25	25.8	26.0
12	40.0*	40.1	26	65.1	65.0
13	40.0*	40.6	27	16.0	16.1
14	56.4	56.3			

*May be reversed.
†Calc. (refs. [6, 7]).

The parent saponin was purified by prep. TLC and recrystallization, $[\alpha]_D^{25}$ −45° (EtOH; *c* 0.5100). The glycosidic portion of the saponin contained glucose, galactose, and xylose as determined by GLC of the aldononitrile acetates [9].

Rhodeasapogenin [10] and isorhodeasapogenin [11], the 5β-analogues of the compound discussed above, have been isolated from *Rhodea japonica*. *Solanum* species are not considered to be rich in nitrogen-free saponins and those that have been found in *Solanum*

generally contain diosgenin as the aglycone, although its epimer, yamogenin and chlorogenin [(25*R*)-5α-spirostan-3β,6α-diol] have been found in a few species. To our knowledge this is the first report of a *Solanum* species that contains a saponin in amounts equivalent to or greater than the amount of glycoalkaloid found in that species [Gregory, P., Sinden, S. L., Osman, S. F. and Chessin, D. A., unpublished observations]. Whether the presence of high levels of this saponin is significant in regard to leaf hopper or Colorado potato beetle resistance remains to be determined. The saponin has been given the trivial name polyadenin and the sapogenin the name polygenin.

EXPERIMENTAL

Mps were obtained on a Fisher–Johns apparatus and are uncorr. Both ^{1}H NMR and ^{13}C NMR spectra were recorded on either a Brucker WH-90 (90 MHz) or JEOL FX60Q (60 MHz) spectrometer in $CHCl_3$ with TMS as int. standard.

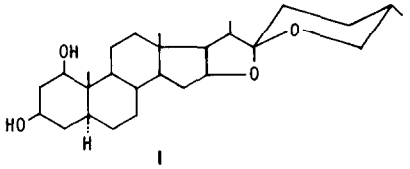
Isolation of polyadenin. Lyophilized leaves of *S. polyadenium* P.I. 161728 (100 g) were extracted with 500 ml 95% EtOH in a Waring blender. The EtOH extracts were concentrated to dryness. The residue was taken up in 150 ml MeOH–H_2O (1:1) and extracted with 50 ml $CHCl_3$. The aq. MeOH was then concentrated and the crude saponin was purified by prep. TLC on Si gel G (500 μm) with $CHCl_3$–MeOH (1:1) as the mobile phase. The product was recrystallized from aq. EtOH; $[\alpha]_D^{25}$ 45° (EtOH; *c* 0.5100).

Isolation of polygenin. Crude 95% EtOH extracts of *S. polyadenium* were concentrated and redissolved in 5% methanolic HCl, the soln was refluxed for 5 hr, concentrated and taken up in $CHCl_3$. The $CHCl_3$ soln was extracted with an equal vol. of 0.1 N H_2SO_4 followed by H_2O extraction. The $CHCl_3$ extracts were chromatographed on Si gel eluting with $CHCl_3$–MeOH (95:5). The partially purified sapogenin was then subjected to prep. TLC on Si gel G (500 μm fluorescent indicator) with $CHCl_3$–MeOH, (97:3) as mobile phase. The sapogenin was recrystallized from hexane–Me_2CO, and EtOH–H_2O; mp 215°, $[\alpha]_D^{25}$ −71° ($CHCl_3$; *c* 0.0670).

Oxidation of polygenin. A soln of 2 mg polygenin in CH_2Cl_2 was pipetted onto a CrO_3–Celite 545 (1:4) column [4]. After 10 min, the column was eluted with CH_2Cl_2 and the product was purified by prep. TLC on Si gel G with $CHCl_3$ used as the mobile phase. The product, $[M]^+$ at *m/z* 428 could be reduced to a mixture of diols via the method of ref. [4].

Dehydration of polygenin. To a soln of 5 ml hexamethylphosphoramide containing 0.5 g methyl-triphenoxyphosphonium iodide [8], 10 mg of polygenin were added. The soln was heated to 75° for 6 hr. After cooling the soln was diluted with H_2O and extracted with Et_2O. The product was purified by TLC (Si gel G, hexane–$CHCl_3$ (1:1)); $[M]^+$ at *m/z* 396; UV $\lambda_{max}^{CHCl_3}$ nm: 262.

Mass spectrum of polygenin TMS. m/z (rel. int.): $[M]^+$ (2), 462 (7), 433 (12), 372 (14), 282 (18), 219 (46), 217 (100), 107 (11), 103 (13), 81 (10), 73 (24), 69 (20), 55 (13).



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REFERENCES

1. Torka, M. (1950) *Am. Potato J.* **27**, 263.
2. Tingey, W. M., Mackenzie, J. D. and Gregory, P. (1978) *Am. Potato J.* **55**, 577.
3. Budzikiewicz, H., Djerassi, D. and Williams, D. H. (1964) In *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, p. 113. Holden-Day, San Francisco.
4. Schwartz, D. P. and Osman, S. F. (1977) *Analyt. Biochem.* **80**, 70.
5. Eggert, H., Van Antwerp, C. L., Bhacca, S. and Djerassi, C. (1976) *J. Org. Chem.* **41**, 71.
6. Van Antwerp, C. L., Eggert, H., Meakins, G. D., Minors, J. O. and Djerassi, C. (1977) *J. Org. Chem.* **42**, 789.
7. Eggert, H. and Djerassi, C. (1975) *Tetrahedron Letters* 3635.
8. Hutchins, R. O., Hutchins, M. G. and Milewski, C. A. (1972) *J. Org. Chem.* **37**, 4190.
9. Varma, R., Varma, R. S. and Wardi, A. N. (1973) *J. Chromatogr.* **77**, 222.
10. Nauva, H. (1953) *Yakugaku Zasshi* **73**, 1193.
11. Mouta, K. (1959) *Bull. Chem. Soc. Jpn* **32**, 791.