

CONVERSION OF DOPA TO TETRAHYDROISOQUINOLINES AND STIZOLOBIC ACID IN A CALLUS CULTURE OF *STIZOLOBIUM HASSJOO*

KOSHI SAITO, HAMAKO OBATA-SASAMOTO,* SHIN-ICHI HATANAKA, HIROSHI NOGUCHI,† USHIO SANKAWA† and ATSUSHI KOMAMINE*

Department of Biology, College of General Education, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan; *Department of Botany, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan; †Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

(Revised received 12 June 1981)

Key Word Index—*Stizolobium hassjoo*; Leguminosae; Yokohama velvet bean; tetrahydroisoquinolines; stizolobic acid; DOPA; callus culture.

Abstract—Isolation and identification of L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and L-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline from seeds and callus of *S. hassjoo* are described. Administration of [β - 14 C]-labelled DOPA to a callus culture of this legume resulted in the incorporation of radioactivity into L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, L-1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and stizolobic acid, which was confirmed by constant specific radioactivity after co-crystallization with authentic samples of each compound.

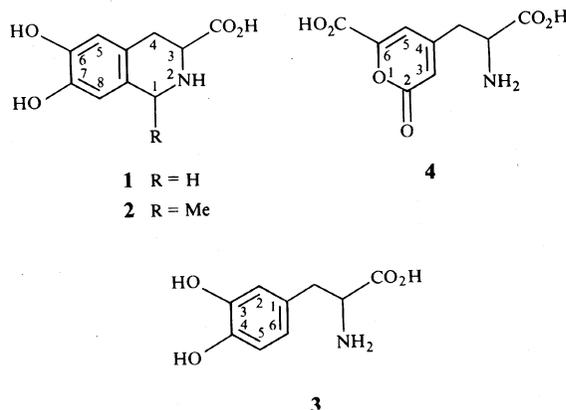
INTRODUCTION

L-3-Carboxy-7, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (**1**) and its C-1 methylated analogue, L-1-methyl-3-carboxy-6, 7-dihydroxy-1, 2, 3, 4-tetrahydroisoquinoline (**2**) have been isolated from seeds of *Mucuna mutisiana* [1] and *M. deeringiana* [2], respectively. On the basis of their structures it can be supposed that these imino acids may arise from DOPA (**3**) by cyclization of the alanyl side-chain to an alicyclic hetero form. As the first step in studying the synthetic mechanism of bicyclic ring systems it is necessary to examine the possibility that these tetrahydroisoquinolines are directly formed from **3**. *Stizolobium hassjoo* is a closely related species to *Mucuna* and also accumulates a large quantity of **3** in seeds and seedlings [3], while only a small amount of **3** was found in a callus culture of *S. hassjoo*. In a previous communication [4], the suppression mechanism for **3** accumulation was investigated in the callus culture of this legume and the active catabolism of **3** was suggested as one possible mechanism. We showed previously that stizolobic acid (**4**) was formed from **3** through an extradiol ring fission followed by recyclization to an α -pyrone ring system [5, 6]. The transformation of **3** to **4** was also reported in a suspension culture of *M. deeringiana* [7]. It is interesting to know whether or not **3** is converted to tetrahydroisoquinolines as well as to **4**. In the present paper, identification of two tetrahydroisoquinolines and the conversion of **3** to these compounds in the callus culture of *S. hassjoo* are described.

RESULTS AND DISCUSSION

2-D PC of EtOH extracts from seeds and callus of *S. hassjoo* revealed the presence of two uncommon

ninhydrin-reacting substances, which were isolated by ion-exchange CC. One gave a bright yellow colour with ninhydrin and the other showed a reddish-brown spot on cellulose TLC. The compounds resembled one another in giving a green colour with FeCl_3 , blackening with $\text{K}_3[\text{Fe}(\text{CN})_6]$ and darkening rapidly in alkaline solution. The elution patterns from a Dowex 1 column were very similar to those of authentic **1** and **2**. Comparison of their chromatographic behaviour on cellulose TLC developed with several different solvents, and their ninhydrin and $\text{K}_3[\text{Fe}(\text{CN})_6]$ coloration, were also in good agreement with those of authentic samples. ^1H and ^{13}C NMR spectrum of **2** isolated from the seeds was coincident with structure **2**. In addition to these tetrahydroisoquinolines a trace amount of **4** was detected by 2-D PC from an EtOH extract of the callus culture.



generally contain diosgenin as the aglycone, although its epimer, yamogenin and chlorogenin [(25*R*)-5 α -spirostan-3 β ,6 α -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 ^1H NMR and ^{13}C NMR spectra were recorded on either a Bruker 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 $\text{MeOH-H}_2\text{O}$ (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 $\text{CHCl}_3\text{-MeOH}$ (1:1) as the mobile phase. The product was recrystallized from aq. EtOH; $[\alpha]_D^{25}$ 45° (EtOH; *c* 0.5100).

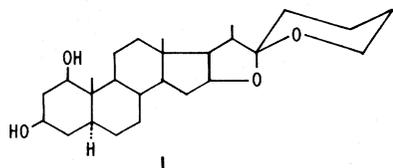
Isolation of polygenin. Crude 95% EtOH extracts of *S. polyadenium* were concentrated and redissolved in 5% methanolic HCl, the soln was refluxed for 5 hr, concentrated and taken up in CHCl_3 . The CHCl_3 soln was extracted with an equal vol. of 0.1 N H_2SO_4 followed by H_2O extraction. The CHCl_3 extracts were chromatographed on Si gel eluting with $\text{CHCl}_3\text{-MeOH}$ (95:5). The partially purified sapogenin was then subjected to prep. TLC on Si gel G (500 μm fluorescent indicator) with $\text{CHCl}_3\text{-MeOH}$, (97:3) as mobile phase. The sapogenin was recrystallized from hexane- Me_2CO , and $\text{EtOH-H}_2\text{O}$; 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 $\text{CrO}_3\text{-Celite 545}$ (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, $[\text{M}]^+$ 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)); $[\text{M}]^+$ at *m/z* 396; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm: 262.

Mass spectrum of polygenin TMS. *m/z* (rel. int.): $[\text{M}]^+$ (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).



Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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1 β -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; 1 β -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 1 β -hydroxyneotigogenin, 5 α -spirostan-1 β ,3 β -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° (CHCl₃; c 0.0670).

High resolution MS showed a molecular ion at m/z 432.3240 corresponding to C₂₇H₄₄O₄. 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-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. ¹³C NMR assignments (Table 1) were based on data in the literature for cholestanols [5], dehydrosteroids [6], and sapogenins [7]. The only structure consistent with all the spectroscopic data is 1 β -hydroxyneotigogenin. The 1,3-substitution was further confirmed by dehydration of the sapogenin

Table 1. ¹³C 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			

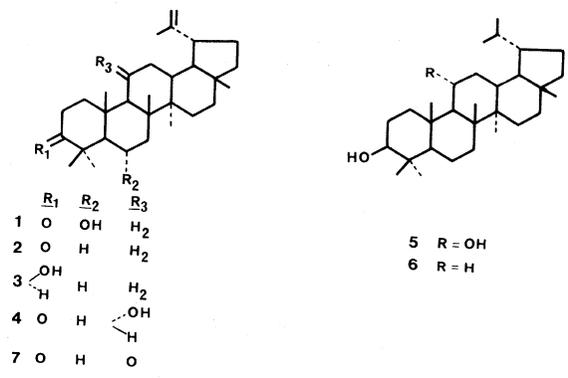
*May be reversed.

†Calc. (refs. [6, 7]).

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 ¹³C NMR spectrum [5, 6] the OHs are diequatorial.

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 aldonitrile acetates [9].

Rhodeasapogenin [10] and isorhodeasapogenin [11], the 5 β -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*



This shift may be attributed to the presence of an equatorial OH group at C-11. In the germanicol series [9] it is also 2.9 and in the steroid derivatives [10] this shift is around 1.4. In comparison with the 11 α -hydroxy-lup-20(30)-en-3-one (4) spectrum, in the 3 β ,11 α -dihydroxy-lupane (5) spectrum the C-12 signal has undergone a downfield shift. This is attributable to the isopropyl configuration in 5 as compared with that which has the isopropylene group in 4 [8].

Finally, we verified by direct comparison that the NMR and IR spectra of the oxidation product of rigidenol and those of 3,11-lupendione (7) [2] were identical. Hence, the structure of rigidenol is 11 α -hydroxy-lup-20(30)-en-3-one (4). Other lupane triterpenes with an OH group at C-11 have been isolated from *Salvia phlomoides* [11] and *Nepeta hindostana* [12].

EXPERIMENTAL

The ¹³C NMR spectra were determined on a Brucker WM 250

at 62.9 MHz and run with the solvent CDCl₃ providing an internal deuterium lock.

Acknowledgements—We wish to thank Dr. I. P. Jones at Brucker Spectrospin, Coventry, for running the ¹³C NMR spectra of 4 and 5, and Professors Jefferies and Ghisalberti, University of Western Australia, for the IR and NMR of 7.

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