Phytochemistry, 1980, Vol. 19, pp. 1249–1251. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0601-1249 $02.00/0

SOLASPIGENIN AND NEOSOLASPIGENIN, TWO NEW SPIROSTANE SAPOGENINS FROM *SOLANUM HISPIDUM**

AJIT K. CHAKRAVARTY, TARUN K. DHAR and SATYESH C. PAKRASHI†

Indian Institute of Experimental Medicine, Jadavpur, Calcutta-700032, India

(*Received* 13 *August* 1979)

Key Word Index—*Solanum hispidum*; Solanaceae; sapogenins; (20*S*,22*S*,23*R*,25*R*)-5α-spirostane-3β,6α,23-triol; (20*S*,22*S*,23*R*,25*S*)-5α-spirostane-3β,6α,23-triol.

We have reported [1] the isolation of hispigenin (**1**), the first naturally occurring 22β-*O*-spirostane sapogenin, along with neochlorogenin (**2**) and paniculogenin (**3**) from *S. hispidum* Pers.‡ The present paper deals with two more new spirostane derivatives, solaspigenin (**4**) and neosolaspigenin (**5**), isolated from the same aglycone mixture obtained by graded Smith degradation [1] of the major birhamnoside fraction of the leaf extract. Neosolaspigenin is the first spirostane encountered in nature with both the C-23 and C-27 substituents axially oriented.

That none of the aglycones was an artifact was ascertained by the absence of a carbonyl band in the IR spectrum of the saponin. Thus, for the isolation of acid-sensitive aglycone in quantitative yield, Smith degradation of saponin appears to be the method of choice where groups reactive to the reagents used are absent.

Because of poor solubility, the mixture of **4** and **5** easily crystallized out of methanol leaving the other co-occurring spirostanes (**1–3**) in solution. The difficult to separate mixture was resolved into the pure components through repeated chromatography of the acetate over Si gel. The homogeneity was ascertained by TLC monitoring of each fraction by multiple elution in the solvent system, C_6H_6–EtOAc (19:1). The sapogenins **4** and **5** were then obtained through alkaline hydrolysis of the respective acetates.

Solaspigenin (**4**), mp > 300°, $[\alpha]_D$ −70.5° ($CHCl_3$–EtOH, 2:3) and neosolaspigenin (**5**), mp 236–240°, $[\alpha]_D$ −70.8° ($CHCl_3$–EtOH, 2:3), both having the formula $C_{27}H_{44}O_5$ (M^+, *m/e* 448), were obtained as white crystalline solids. Both showed IR bands at 983, 960, 936, 910 and 870 cm^{-1}, characteristic [2] of a spirostane ring system, besides broad bands between 3200 and 3500 cm^{-1} for OH group(s).

With Ac_2O–Py at room temperature, **4** and **5** formed the respective triacetates, **6** (M^+, *m/e* 574), mp 174–176°, $[\alpha]_D$ −59.37° ($CHCl_3$), ν_{max}^{nujol} 1735, 1240, 968, 926, 912, 905, 882 cm^{-1}, and **7** (M^+, *m/e* 574), mp 194–196°, $[\alpha]_D$ −61° ($CHCl_3$), ν_{max}^{nujol} 1735, 1240, 975, 932, 904, 873 cm^{-1}. The absence of any OH band in the IR spectrum of the acetates indicated that both **4** and **5** were trihydroxy spirostanes and that all the hydroxyl functions were unhindered.

That both the sapogenins were 3,6,23-trihydroxy spirostane derivatives was apparent from their MS fragmentation patterns [1, 3, 4] which were identical with those of hispigenin (**1**) and paniculogenin (**3**), exhibiting peaks at *m/e* 363 (base peak), 345, 327, 289, 271 and 253. The structure and stereochemistry of the sapogenins, however, were mainly established from the ^1H NMR spectra of the triacetates **6** and **7**.

The identical chemical shift (Table 1) of 19-Me of the acetates and that of paniculogenin triacetate (**8**) [1] indicated [5] the presence of a 3β, 6α-diacetoxy-5α-androstane moiety in both **6** and **7**. The signals for 23-H at δ 4.83 in **6** and 4.80 in **7** as multiplets with $W_{1/2}$ = 6–7 Hz showed that the 23-OAc groups in both the compounds must be axial. This was also supported by the downfield shift of the 21-Me signal to δ 1.03 in **6** and 1.06 in **7** compared to 0.98 in **8** (*loc. cit.*). Since no deshielding of 18-Me was observed in relation to **8**, 21-Me must be α-oriented [6, 7] in both the compounds.

That solaspigenin (**4**) and neosolaspigenin (**5**) differ with respect to the stereochemistry at C-25 (*R* in **4** and *S* in **5**) was inferred from the characteristic [8, 9] chemical shift of 27-Me and the shift and splitting pattern of 26-H_2. Thus, 27-Me was found to be more deshielded in **7** compared to that in **6**. Furthermore, the signal for 26-H_2 was observed as a multiplet at δ 3.50 ($W_{1/2}$ = 6 Hz) in **6** while in **7** it consisted of a doublet at 3.36 along with a double doublet at 4.06.

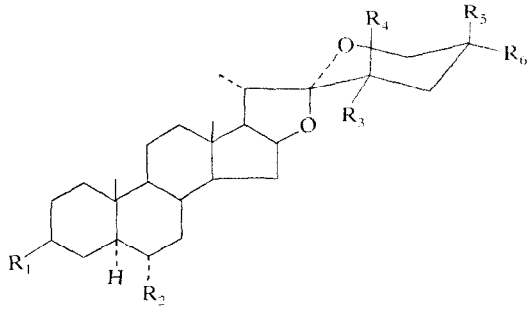
On Jones oxidation, neosolaspigenin (**5**) yielded a triketone **9**, mp 242–244°, identical (IR) with that obtained from paniculogenin (**3**), while solaspigenin (**4**) afforded a different triketone **10**, mp 236–240°. The ^1H NMR spectra (Table 1) of the ketones **9** and **10** exhibited chemical shifts for 21-Me, 27-Me and 26-H_2 in good agreement with the data [3, 10] for (25*S*)- and (25*R*)-23-ketospirostanes, respectively. It may be pointed out that the signal for an equatorial 27-Me undergoes a downfield shift of *ca* 0.15 ppm on changing a 23-axial OAc to a 23-oxo group, both in the case of hispigenin (**1**) and solaspigenin (**4**).

The correlation of neosolaspigenin (**5**) with

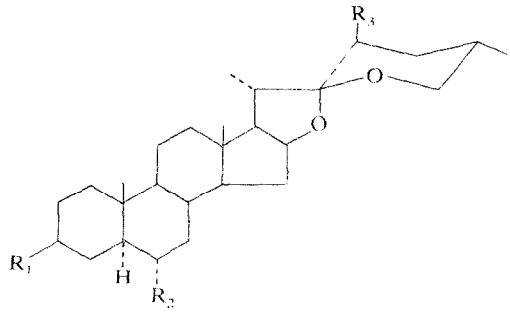
* Part 55 in the series "Studies on Indian Medicinal Plants". For Part 54 see Chakravarty, A. K., Saha, C. R. and Pakrashi, S. C. (1979) *Phytochemistry* **18**, 902.

† To whom all correspondence should be addressed.

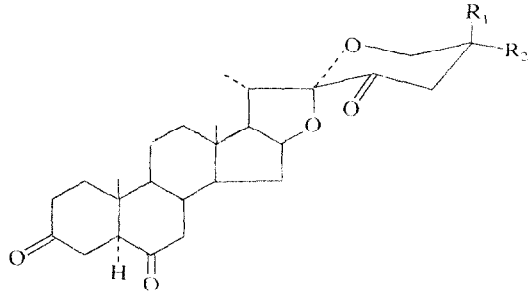
‡ The plant material was collected from Dehradun, U. P. during February–March by M/S. United Chemicals & Allied Products, Calcutta, and a voucher specimen (No. 1236) is available at the herbarium of the supplier.



2 $R_1 = R_2 = OH$, $R_3 = R_4 = R_6 = H$, $R_5 = Me$
3 $R_1 = R_2 = R_3 = OH$, $R_4 = R_6 = H$, $R_5 = Me$
4 $R_1 = R_2 = R_4 = OH$, $R_3 = R_5 = H$, $R_6 = Me$
5 $R_1 = R_2 = R_4 = OH$, $R_3 = R_6 = H$, $R_5 = Me$
6 $R_1 = R_2 = R_4 = OAc$, $R_3 = R_5 = H$, $R_6 = Me$
7 $R_1 = R_2 = R_4 = OAc$, $R_3 = R_6 = H$, $R_5 = Me$
8 $R_1 = R_2 = R_3 = OAc$, $R_4 = R_6 = H$, $R_5 = Me$



1 $R_1 = R_2 = R_3 = OH$
11 3,6,23-Triketo



9 $R_1 = Me$, $R_2 = H$
10 $R_1 = H$, $R_2 = Me$

paniculogenin (**3**) unequivocally establishes the 22α-*O*-stereochemistry in the former. The same stereochemistry at C-22 could also be assigned to solaspigenin (**4**) from the close correspondence of the optical rotations [1, 11] of the new sapogenins (**4** vs **5**) and their acetates (**6** vs **7**). It was further supported [1, 9] by the resonance frequency of 16-H (Table 1) of solaspigenin trione (**10**).

Thus, solaspigenin and neosolaspigenin may respectively be represented by (20*S*,22*S*,23*R*,25*R*)- and (20*S*,22*S*,23*R*,25*S*)5α-spirostane-3β,6α,23-triol, structures **4** and **5**.

Acknowledgement—The authors are grateful to C.S.I.R., New Delhi, for awarding a Senior Research Fellowship to one of them (T. K. D.).

REFERENCES

1. Chakravarty, A. K., Dhar, T. K. and Pakrashi, S. C. (1978) *Tetrahedron Letters* 3875.
2. Jones, R. N., Katzenellenbogen, E. and Dobriner, K. (1953) *J. Am. Chem. Soc.* **75,** 158.
3. González, A. G., Freire, R., Garcia-Estrada, M. G., Salazar, J. A. and Suarez, E. (1972) *Tetrahedron* **28,** 1289.

Table 1. ^{1}H NMR data (δ) of derivatives of sapogenins in $CDCl_3$*

Compound	19-Me	18-Me	21-Me	27-Me	26-H_2	16-H	3-H and 23-H	3-H and 6-H	OAc
6	0.90	0.76	1.03 *d* (7)	0.76 *d* (7)	3.50 *m*	4.53 *m*	4.83 *m*	4.53 *m*	2.03, 2.10
7	0.90	0.73	1.06 *d* (7)	1.19 *d* (7)	3.36 *d*(11), 4.06 *dd*(11, 3)	4.50 *m*	4.80 *m*	4.50 *m*	2.03, 2.06
9	0.98	0.82	0.98 *d* (7)	1.07 *d* (7)	3.40 *d*(12), 4.25 *dd*(12, 3)	4.65 *m*	—	—	—
10	0.97	0.80	0.93 *d* (6)	0.93 *d* (6)	3.63 *m*	4.66 *m*	—	—	—
11†	0.96	0.86	1.05 *d* (6)	0.96 *d* (6)	3.72 *d*(9)	4.15 *ddd* (7, 7, 7)	—	—	—

* Spectra taken in a 90 MHz instrument. The figures in parentheses are coupling constants in Hz.

† Correct assignments of the signals of hispigenin trione (**11**). The chemical shifts of 21-Me and 27-Me were inadvertently reversed in our previous publication [1].

4. Faul, W. H. and Djerassi, C. (1970) *Org. Mass Spectrom.* **3,** 1187.
5. Tori, K. and Aono, K. (1964) *Ann. Rep. Shionogi Res. Lab.* **14,** 136.
6. Green, G. F. H., Page, J. E. and Staniforth, S. E. (1966) *J. Chem. Soc. B* 807.
7. Boll, P. M. and Philipsborn, W. V. (1965) *Acta Chem. Scand.* **19,** 1365.
8. Kutney, J. P. (1963) *Steroids* **2,** 225.
9. Williams, D. H. and Bhacca, N. S. (1965) *Tetrahedron* **21,** 1641.
10. Freire, R., González, A. G. and Suarez, E. (1970) *Tetrahedron* **26,** 3233.
11. Wall, M. E. (1955) *Experientia,* **11,** 340.

Phytochemistry, 1980, Vol. 19, pp. 1251–1252. Pergamon Press Ltd. Printed in England.

UN NOUVEL HETEROSIDE NITRÉ EXTRAIT D'*ANNONA SQUAMOSA*

P. FORGACS, J. F. DESCONCLOIS, J. PROVOST, R. TIBERGHIEN et A. TOUCHÉ

Centre de Recherches des Laboratoires Roger Bellon, 90 rue M. Bourdarias, 94140-Alfortville, France

(*Recu le* 14 *septembre* 1979)

Key Word Index—*Annona squamosa*; Annonaceae; primeveroside; 4-(-2-nitroethyl)phenol; 4-(-2 aminoethyl)phenol; tyramine.

Abstract—A new glycoside was isolated from a 60% methanol extract of dried leaves and stems of *Annona squamosa*. Its chemical structure was determined as 4-(-2-nitroethyl)-1-[(6-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy] benzene.

INTRODUCTION

A côté des alcaloïdes aporphiniques déjà identifiés dans un extrait hydro-éthanolique des feuilles et tiges d'*Annona squamosa* L. récoltées en Inde [1], a été isolé un nouveau constituant hétérosidique, possédant un groupe nitré.

Les dérivés nitrés sont peu répandus parmi les plantes supérieures. Le principe odorant des huiles essentielles de *Dennettia tripetala*, G. Baker (Annonacées) est le 1-nitro 2-phényl éthane [2]. Ce même corps a été trouvé dans les huiles tirées des écorces d'*Aniba canellia* (Lauracées) [3]. D'autres composés nitrés sont extraits des plantes, en particulier, l'acide aristolochique et l'acide hiptagénique (ou acide β-nitro propionique) d'*Aristolochia clematitis* (Artistolochiacées) [4]. Des esters glucosidiques de l'acide β-nitro propionique sont décrits dans diverses familles de plantes, comme *Corynocarpus laevigata* (Corynocarpacés), *Hiptage madablata* (Malpighiacées), *Indigofera endecaphylla* (Légumineuses) [5] et *Coronilla varia* (Légumineuses) [6].

RÉSULTATS ET DISCUSSION

Les feuilles et tiges séchées d'*Annona squamosa* L. sont extraites par le mélange méthanol–eau (3:2). L'isolement du produit (**1**), repéré en CCM est effectué par une adsorption sur Amberlite XAD_2 [7], suivie d'une élution sur colonne de polyamide et d'une chromatographie sur gel de Si. Le composé est cristallisé dans l'éthanol F 125°; $[\alpha]_D^{20} - 70°$ (EtOH, *c* 2). Il répond à la formule de $C_{19}H_{27}NO_{12}$. Son spectre IR dans le KBr présente des massifs à 3300 et 1050 cm^{-1}, caractéristiques de groupements polyhydroxylés, ainsi qu'une bande à 1385 cm^{-1} supposant l'existence de $—NO_2$. L'hydrolyse de (**1**) dans l'acide sulfurique 2 N donne deux sucres: le xylose et le glucose et un aglycone (**2**): $C_8H_9NO_3$. On observe un seul pic en CPG et une seule tache en CCM. Par la réduction catalytique de ce dernier, sur Pd/C à 5% dans l'éthanol on obtient quantitativement la tyramine ou le 4-(-2-aminoéthyl) phénol (**3**). L'aglycone (**2**) est identifié au 4-(-2-nitroéthyl) phénol, produit décrit par synthèse [8].