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SOLAPLUMBIN, A NEW ANTICANCER GLYCOSIDE FROM *NICOTIANA PLUMBAGINIFOLIA**

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Key Word Index—*Nicotiana plumbaginifolia*; Solanaceae; solaplumbin; solaplumbinin; solasodine glycoside.

Plant. Nicotiana plumbaginifolia Viv. a weed native to Mexico, West Indies and naturalized in India.[1] *Source.* Locally collected by Botany Division of the Institute. *Previous work.* Anticancer activity in Walker carcinosarcoma 256 system;[2] nornicotine.[3] *Present work.* The concentrate of the alcoholic extracts of the air dried, above ground parts of the plant was fractionated successively with petrol., $CHCl_3$ *n*-BuOH and H_2O. The anticancer activity was found to be confined to the *n*-BuOH fraction. Chromatography over silica gel using MeOH:EtOAc (1:9) as eluents, yielded the active principle, named as solaplumbin (**1**), in a yield of 0·1% solaplumbin, m.p. 180–181°, $(\alpha)_D^{20}$ −90° gave positive tests for glycoside and alkaloid. It analysed for $C_{39}H_{63}O_{11}N$ and formed a hexaacetate, $C_{51}H_{75}O_{17}N$, m.p. 152–154°. On acid hydrolysis, solaplumbin gave solasodine,[4] identified by m.p., m.m.p., U.V., I.R., NMR,[5] MS[6] and preparation of conventional derivatives, while the sugars were identified as glucose and rhamnose by co-PC. The molar ratio of sugars was quantitatively estimated as 1:1 by colorimetry through paper chromatography. The presence of 1 mole each of glucose and rhamnose in the solaplumbin molecule was also evident from the NMR spectrum of the acetylated glycoside which showed the presence of six acetyl groups at δ 1·95–2·05 ppm.

Enzymatic hydrolysis of solaplumbin with takadiastase liberated glucose, indicating it to be the end sugar, β-linked through C-1, and a partial glycoside (**2**). Purification of the latter by column chromatography gave a TLC-pure new glycoside named solaplumbinin (**2**), m.p. 184–185°, $(\alpha)_D^{20}$ −39·5°. It analysed for $C_{33}H_{53}O_6N$ and reduced Fehling's solution and aniline–hydrogen phthalate on PC. On acid hydrolysis, **2** gave only rhamnose and solasodine. Thus **2** was rhamnoside of solasodine. In order to fix the position of the linkage in **2**, periodate oxidation studies were carried out with both **1** and **2**. In each case, one mole of formic acid was generated. In case of **1**, obviously it came from the glucose part where three vicinal hydroxyl groups were free while in case of **2** it suggested that three vicinal hydroxyl groups of rhamnose were also free and therefore, the latter (rhamnose) was linked to solasodine either through position 1 or 4. Since **2** was reducing in nature,

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the linkage of rhamnose to solasodine could not be through 1 and the alternative position was, therefore 4. This established the structure of **2** as rhamnosyl (4 → 3) solasodine, leading to solaplumbin (**1**) as β-D-glucosyl (1 → 1) β-L-rhamnosyl (4 → 3) solasodine. Further support for this structure was obtained when permethyl solaplumbin $C_{45}H_{75}O_{11}N$, m.p. 138–142° prepared by NaH/MeI followed by acid hydrolysis, yielded 2,3,4,6-tetramethyl glucose and 2,3-di-*o*-methyl rhamnose identified by co-GLC with authentic samples.

The anticancer activities of both the glycosides **1** and **2** are given in Table 1.

TABLE 1

Compd.	Dose (mg)	T/C*	Survival
Solaplumbin **1**	15	13	4/6
	12·5	35	4/6
	7·5	43	6/6
Solaplumbinin **2**	20	11	6/6
	10	17	6/6
	5	70	6/6

* T/C = treated/Control, Values below 48 show compound to be active.

EXPERIMENTAL

M.ps are uncorrected, IR spectra were taken in KBr and 60 Mcs NMR in $CDCl_3$ with tetramethylsilane as internal reference. $(\alpha)_D$ are given for 1% solution in MeOH at 20°. GLC was carried out on a Varian SE30 column, 1·5 m × 3 mm, injection temp. 225°, column temp. 150°. TLC was on silica gel plates and PC at room temp. on Whatman No. 1 paper. System I: *n*-BuOH–HOAc–H_2O (4:1:5), upper; II EtOAc–pyridine–H_2O(2:1:2) descending. Non-aq. solns were dried and anhyd. sodium sulphate.

Isolation of solaplumbin (**1**). Air dried, finely powdered aerial plant material (10 kg) was extracted with 95% EtOH (10 lit. × 5) at room temp. The combined extracts were conc. *in vacuo* at a temp. below 45° and the resulting thick green paste (1 kg) was extracted successively with C_6H_{14}, $CHCl_3$, *n*-BuOH and H_2O. Of these only the *n*-BuOH fraction (50 g) showed anticancer activity (dose 50 mg, T/C. 16, survival 6/6). Chromatography of this fraction (silica gel column) and successive elution with C_6H_6, C_6H_6–EtOAc (1:1), EtOAc, MeOH: EtOAc (1:9), localized the anti-cancer activity in the MeOH: EtOAc (1:9) eluted fraction. The residue responded to positive Feigel test for glycoside and positive Dragendorff test for alkaloid. Recrystallization (EtOH–C_6H_6) gave pale yellow needles, of solaplumbin (**1**), m.p. 180–181° $(\alpha)_D$ −90°, TLC R_f 0·24, [MeOH–EtOAc (1:4)], IR ν max. 3330, 2900, 1680 (weak), 1460, 1380, 1150–1000 (broad) etc. cm^{-1}. (Found C, 64·7; H, 9·1; N, 1·7; reqd. for $C_{39}H_{63}O_{11}$, C, 64·9; H, 8·7; N, 1·9%).

Acid hydrolysis of the solaplumbin (**1**). Solaplumbin (1·5 g) was treated with 10% methanolic HCl (10 ml) under reflux for 4 hr. The white crystalline material separated as solasodine HCl, which on basification with NH_4OH yielded colourless crystals of solasodine, m.p. 197–198°.

Acetylation of solaplumbin. Solaplumbin (100 mg) on acetylation with Ac_2O (1 ml), yielded hexaacetyl solaplumbin (90 mg) m.p. 152–154°, IR ν_{max} 1735 and 1250 cm^{-1}, NMR: δ 0·85-*O*·92 (5 × Me), 1·2 (broad signal for 28 proton of methine and methylene), 1·95–2·05 (6 × OCOMe), 3·6 (broad 3H, adjacent to *N* and *O*), 4·7–5·1 (broad multiplet 9H), 5·2 (1H olefinic proton). (Found C, 62·7; H, 7·8; N, 1·34 reqd. for $C_{51}H_{75}O_{17}N$; C, 62·89; H, 7·70; N, 1·43%).

Enzymatic hydrolysis of solaplumbin (**1**): *Solaplumbinin* (**2**). Solaplumbin (200 mg) was hydrolysed with takadiastase (100 mg) at 37° for 60 hr. The residue was partitioned between *n*-BuOH (20 ml) and H_2O. The aq. layer on co-PC showed glucose. The *n*-BuOH layer gave a brown mass which on chromatography over silica gel, and elution with MeOH:EtOAc (1:9) gave solaplumbinin (**2**) as a white amorphous powder, m.p. 184–185°, $(\alpha)_D$ −39·5°, (TLC R_f 0·4 [EtOAc–MeOH (9:1)]. (Found, C, 70·6; H, 9·8; N, 2·35; reqd. for $C_{33}H_{53}O_6N$, C, 70·8; H, 9·4; N, 2·5%).

Examination of the sugar fractions of **1** *and* **2**. The aq. hydrolysate was examined by co-PC in solvent systems **1** and **2**, identifying sugars as rhamnose and glucose in **1** and only rhamnose in **2**.

Methylation of solaplumbin. Solaplumbin (500 mg) in dry DMSO (10 ml) was stirred with NaH (500 mg) and CH_3I (2·5 ml) overnight in the dark. The usual working and chromatography over silica gel yielded methyl solaplumbin (200 mg), TLC R_f 0·8 [$CHCl_3$:MeOH (9:1)], m.p. 138–142°C, (Found: C, 66·9; H, 9·5; N, 1·6; reqd. for $C_{45}H_{75}O_{11}N$, C, 67·08; H, 9·3; N, 1·73%).

Acid hydrolysis of methyl solaplumbin. Methyl solaplumbin (150 mg) was refluxed with 10% H_2SO_4 in 50% MeOH (10 ml) for 4 hr. Methyl sugars were examined by CO–GLC as 2,3,4,6-tetramethyl glucose and 2,3-dimethyl rhamnose.

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PELARGONIDIN IN THE FLOWERS OF A MUTANT OF *PETUNIA HYBRIDA*

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Key Word Index—*Petunia hybrida*; Solanaceae; anthocyanins; flavonols; pelargonidin; kaempferol.

The flowers of *Petunia hybrida* and its numerous horticultural forms contain many different anthocyanins[1-3] but, so far, glycosides of pelargonidin have not been found. Mutagenic treatments[4] applied to cyanidin and quercetin lines yielded carriers of a recessive mutation (*k*) which promotes the synthesis of kaempferol at the expense of quercetin. The mutant gene *k* also seems to be responsible for a decrease in anthocyanin production.

By selection of these "kaempferol" lines, we have obtained plants containing a mixture of the 3-monoglucosides of pelargonidin, cyanidin and paeonidin. The different lines do not synthesize the same relative quantities of pelargonidin: in the best case, pelargonidin is produced in approximately the same amount as the other two pigments. However, pelargonidin carriers cannot be identified through flower color. It is hoped that selection will provide flowers with higher pelargonidin content which could lead to new color varieties of *Petunia*.

EXPERIMENTAL

The anthocyanins were extracted from the petals by grinding in MeOH + 1% HCl. The anthocyanidins were separated by 2-D cellulose TLC,[5] after acid hydrolysis of the anthocyanins, in HCl–HCOOH–H_2O (1:10:1) and AmOH–HOAc–H_2O (2:2:1). The anthocyanins were identified by PC. Pelargonidin glycoside was purified in BAW (2x) BuOH–HCl 2 N, 1% HCl, HOAc–HCl.[6] A pelargonidin-3-glucoside marker was obtained by partial hydrolysis of pelargonidin-3,5-diglucoside extracted from petals of the rose variety "Miss France".[7] Flavonols were identified by cellulose TLC after acid hydrolysis in HCl–HCOOH–H_2O (1:10:3).

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