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A NOR-SESQUITERPENE GLYCOSIDE, RISHITIN-β-SOPHOROSIDE, FROM TOBACCO*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; rishitin-β-sophoroside.

Abstract—A new nor-sesquiterpene glycoside, isolated from flue-cured tobacco, was identified as rishitin-β-sophoroside. The absolute configuration of the aglycone, rishitin, was identical with that obtained from potato tuber tissue infected by pathogens.

INTRODUCTION

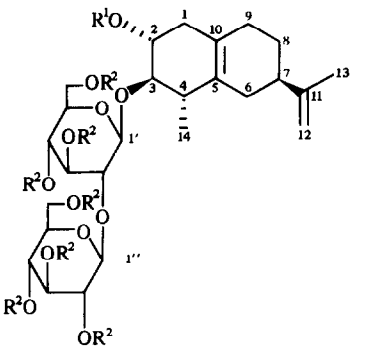
Our research on the non-volatile constituents of tobacco leaves has resulted in the isolation and structural characterization of several glucosides of ionone-related compounds [1–3]. Four vetispirane sesquiterpene glucosides have been isolated from tobacco leaves by Anderson *et al.* [4]. Recently, the presence of rishitin, which was first isolated from potato tuber tissue infected by pathogens [5, 6] in essential oil of flue-cured tobacco leaves, was reported [7]. In this paper, we describe the isolation of rishitin-β-sophoroside from flue-cured tobacco leaves.

RESULTS AND DISCUSSION

Successive fractionation or methanol extracts of flue-cured tobacco by solvent partition, charcoal and silica gel chromatography, and HPLC (μ-bondapak C_{18}, 55% MeOH–H_2O) resulted in the isolation of the glycoside **1**. Enzymatic hydrolysis of this glycoside with β-glucosidase liberated an aglycone which was purified by HPLC (μ-porasil, 20% EtOAc–$CHCl_3$) to a single peak on capillary GC (OV-101, 40 m, 100–240°, 2°/min). The spectral and physical data of the aglycone were identical with those of rishitin in the literature [8]. The absolute configuration of the rishitin was established by the identical nature of $[\alpha]_D$ and the ^{13}C NMR spectrum (Table 1) to those in the literature.

Glycoside **1** was treated with acetic anhydride–pyridine and successively saponified with ammonia–methanol. The impurities were removed by HPLC (μ-bondapak C_{18}, 55% MeOH–H_2O), in which the retention time of reaction product **2** was not identical with that of glycoside **1**. In our previous report, saponification of peracetylated 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside by ammonia–methanol gave rise to only 5,6-epoxy-5,6-dihydro-3-acetoxy-β-ionyl-β-glucoside [1]. This suggested that an acetyl group would remain in the aglycone on similar treatment of glycoside **1**. On FDMS of **2**, the $[M+Na]^+$ cluster ion at m/z 611 suggested its structure consisted of a hexabiose and rishitin monoacetate. Sugar composition analysis by the dithioacetal-TMSi method [9] showed that the sugar moiety of **2** was made up of two molecules of glucose which were shown to be β1-2 linked by methylation analysis. Furthermore, a striking feature of the ^{13}C NMR spectrum of the sugar moiety of **2** is its close similarity with the corresponding data for methyl-β-sophoroside [10] (Table 1). On the basis of these chemical and spectral data, the sugar moiety of **2** was assigned to be β-sophoroside.

In the early stage of the treatment of glycoside **1** with acetic anhydride–pyridine at 0°, two spots were seen on TLC [R_f 0.56 and 0.48, $CHCl_3$–EtOAc (3:2)]. Preparative HPLC (μ-bondapak C_{18}, 70% MeOH–H_2O) afforded compound **3** corresponding to the spot at R_f 0.48 and compound **4** corresponding to the spot at R_f 0.56. In the FDMS of **3**, the $[M+H]^+$ cluster ion at m/z 841 showed that one hydroxyl group was not acetylated in



	R^1	R^2
1	H	H
2	Ac	H
3	H	Ac

*Part 3 in the series "Non-volatile Constituents in Tobacco". For Part 2 see ref. [2].

Table 1. ^{13}C NMR spectra (25.15 MHz, $CDCl_3$, TMS as int. standard)

C	Rishitin [8]	Rishitin (aglycone)	2	3	Methyl-β-sophoroside [10]
1	26.5	26.6	27.7	26.6	
2	71.5	71.5	71.5	69.0	
3	79.2	79.3	81.9	93.4	
4	40.4	40.5	39.2	40.5	
5	129.0	129.1	129.3	128.6	
6	31.1	31.1	32.7	31.1	
7	41.6	41.7	41.9	40.5	
8	38.3	38.4	33.1	37.7	
9	29.7	29.7	30.3	29.6	
10	124.9	124.8	123.2	124.7	
11	148.9	148.9	*	148.8	
12	109.0	109.0	109.4	108.9	
13	21.0	21.1	20.9	20.5	
14	16.4	16.4	17.9	16.4	
1′			103.9	100.7	103.7
2′			84.0	77.4	83.5
3′			78.5	72.9	78.6
4′			70.8	68.3	71.2
5′			78.0†	71.5	78.3
6′			62.8	61.8	62.5
1″			106.2	102.3	106.0
2″			76.5	71.2	76.4
3″			78.5	74.8	78.9
4″			71.5	68.6	71.5
5″			78.0†	71.7	78.3
6″			62.8	61.8	62.5

*Under solvent.
†These assignments may be reversed.

glycoside **1**. The EIMS of **3** showed $[M]^+$ at *m/z* 840 and the fragment peaks of heptaacetylated hexabiose and tetraacetylated hexose at *m/z* 619 and 331, respectively. This suggested that the sugar moiety of **3** was peracetylated β-sophoroside and the hydroxyl group of the aglycone, rishitin, was not acetylated. The ^1H NMR spectrum of **3** showed the characteristic signals of rishitin at δ1.21 (H-14), 1.73 (H-13), 3.17 (H-3) and heptaacetylated β-sophoroside at 4.53 (anomeric proton) and 4.68 (anomeric proton). In the ^{13}C NMR spectrum of **3**, the chemical shifts of aglycone carbons were almost identical with those of rishitin, except for the signal at δ93.4. On the basis of an analysis of the selective ^1H decoupled ^{13}C NMR spectrum of **3**, the signal at δ93.4 was assigned to C-3 by irradiation of the H-3 proton (δ3.17, ^1H NMR spectrum). The signal assigned to C-3 was shifted by +14.2 ppm in comparison with that of rishitin by the glycosidation shift [11] (Table 1). Thus, the linkage position of the sophorose unit was confirmed to be at C-3 of rishitin. Compound **4** (R_f 0.56) was thought to be peracetylated glycoside **1**, because **3** was converted to the compound **4** by further treatment with acetic anhydride–pyridine at room temperature. Based on the above evidence, glycoside **1** is rishitin-β-sophoroside.

EXPERIMENTAL

HPLC was carried out on μ-bondapak C_{18} (Waters, 3.9 mm i.d. × 30 cm) and μ-porasil (Waters, 3.9 mm i.d. × 30 cm) using a Waters solvent delivery system (M 6000 A constant flow pump and R401 differential refractometer). ^1H and ^{13}C NMR spectra were recorded with JEOL FX-100 (int. standard TMS).

The procedures for the extraction and fractionation of flue-cured tobacco leaves (3 kg) were described in the previous communication [1]. The MeOH eluate from CC on charcoal (1.2 g) was successively fractionated on silica gel. The fraction eluted with 20% MeOH–$CHCl_3$ (240 mg) was purified by HPLC (μ-bondapak C_{18}, 55% MeOH–H_2O). Glycoside **1** was obtained as a colourless oil.

Sugar composition analysis. This was carried out by the method of ref. [9]. The dimethyl dithioacetal-TMSi derivative was analysed by comparison of GC R_t with standards (capillary OV-101, 40 m, 220° isothermal).

Methylation analysis. This was carried out by the method of refs [12] and [13]. The partially methylated alditol acetates were analysed by capillary GC/MS.

Rishitin. $[\alpha]_D = -33.0°$ (*c* 0.405: EtOH); EIMS 70 eV, *m/z* (rel. int.): 222 $[M]^+$ (11), 204 (59), 161 (70), 143 (100), 131 (58), 119 (74), 107 (48); ^1H NMR (100 MHz, $CDCl_3$): δ1.16 (3H, *d*, *J* = 6.6 Hz, H-14), 1.74 (3H, *s*, H-13), 3.23 (1H, *t*, *J* = 9.0 Hz, H-3), 3.66 (1H, *dt* (*br*), *J* = 8 and 9 Hz, H-2), 4.64 (2H, *s* (*br*), H-12), 4.75 (2H, *s* (*br*), OH); ^{13}C NMR (25.15 MHz, $CDCl_3$): Table 1.

3-Acetoxy-rishitin-β-sophoroside (**2**). ^1H NMR (100 MHz, C_5D_5N): δ1.28 (3H, *d*, *J* = 7.3 Hz, H-14), 1.70 (3H, *s*, H-13), 2.04 (3H, *s*, OAc), 4.79 (2H, *s* (*br*), H-12), 5.06 (1H, *d*, *J* = 7 Hz, H-1′ or 1″), 5.23 (1H, *d*, *J* = 6 Hz, H-1′ or 1″). ^{13}C NMR (25.15 MHz, $CDCl_3$): Table 1; FDMS: see text.

Rishitin-β-sophoroside heptaacetate (**3**). ^1H NMR (100 MHz, $CDCl_3$): δ1.21 (3H, *d*, *J* = 6.3 Hz, H-14), 1.73 (3H, *s*, H-13), 3.17 (1H, *d*, *J* = 8.8 Hz, H-3); EIMS 70 eV, *m/z* (rel. int.): 840 $[M]^+$ (< 0.1), 619 (27), 331 (100), 204 (29), 169 (25); ^{13}C NMR (25.15 MHz, $CDCl_3$): Table 1; FDMS: see text.

Rishitin-β-sophoroside (**1**). ^1H NMR (100 MHz, C_5D_5N): δ1.53 (3H, *d*, *J* = 6.8 Hz, H-14), 1.72 (3H, *s*, H-13), 3.66 (1H, *t*, *J* = 8.5 Hz, H-3), 5.03 (1H, *d*, *J* = 7.1 Hz, H-1′ or 1″).

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UROSPERMAL, A GLUCOSIDE FROM *UROSPERMUM PICROIDES*

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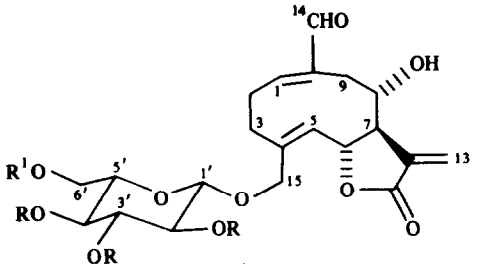
Key Word Index—*Urospermum picroides*; Compositae; sesquiterpene lactones; urospermal A glucoside.

Abstract—The roots of *Urospermum picroides* afforded urospermal A 15-*O*-β-D-glucoside.

The aerial parts of *Urospermum picroides* (L.) Scop. ex F. W. Schmidt contain the melampolide urospermal A and the *p*-hydroxyphenyl acetate of the corresponding glucoside [1]. We have now studied the polar fractions from the roots. Thin-layer chromatography afforded two compounds, the glucosides **1** and **2**. Acetylation gave the acetates **3** and **4**, the latter being identical with the acetylation product of the glucoside **2** isolated from the aerial parts [1]. The ^{1}H NMR spectrum of **3** (Table 1) was close to that of **4**. However, the signals of the *p*-hydroxyphenyl acetate were replaced by an additional acetate methyl signal. As observed previously [1] the hydrogen-bonded 8α-hydroxyl group was not acetylated. The ^{1}H NMR spectrum of the natural compound **1** could be measured only in deutero-pyridine (Table 1). All signals were assigned by spin decoupling in the usual way, starting with the H-7 signal although all signals were



1 R = R^1 = H
2 R = H, R^1 = $COCH_2C_6H_4OH$ (p)
3 R = R^1 = Ac
4 R = Ac, R^1 = $COCH_2C_6H_4OAc$ (p)

Table 1. ^{1}H NMR spectral data of compounds **1** and **3** (400 MHz, TMS as internal standard)

	1 (C_5D_5N)	**3** ($CDCl_3$)
H-1	6.67 *br dd*	6.81 *br dd*
H-2	2.53 *ddd*	2.54 *m*
H-2′	2.26 *ddd*	
H-3	2.68 *ddd*	2.61 *ddd*
H-3′	1.80 *br dd*	2.03 *m*
H-4	5.15 *br d*	5.15 *br d*
H-6	4.98 *dd*	4.57 *dd*
H-7	2.65 *dddd*	2.45 *dddd*
H-8	4.27 *ddd*	3.90 *dddd*
H-9	2.98 *dd*	2.70 *dd*
H-9′	2.44 *br d*	2.37 *br d*
H-13	6.60 *dd*	6.52 *dd*
H-13′	6.33 *dd*	6.30 *dd*
H-14	9.49 *br s*	9.44 *br s*
H-15	4.77 *d*	4.40 *d*
H-15′	4.44 *d*	4.33 *d*
H-1′	4.91 *d*	4.58 *d*
H-2′	4.00 *dd*	5.04 *dd*
H-3′	4.20 *m*	5.22 *dd*
H-4′		5.08 *dd*
H-5′	3.95 *m*	3.73 *dd*
H-6′$_1$	4.54 *dd*	4.26 *dd*
H-6′$_2$	4.36 *dd*	4.17 *dd*
OH	6.06 *d*	6.07 *d*
OAc	—	2.08 *s*
		2.03 *s*
		2.02 *s*
		1.98 *s*

J (Hz): 1, 2 = 9; 1, 2′ = 8; 1, 9 ~ 1; 5, 6 = 6, 7 = 7, 8 = 10; 7, 13 = 3.5; 7, 13′ = 3; 8, OH = 11, 5; 8, 9 = 5; 8, 9′ = 11; 9, 9′ = 16; 13, 13′ = 2; 15, 15′ = 12; 1′, 2′ = 8.5; 2′, 3′ = 3′, 4′ = 4′$_1$, 5′ = 9.5; 5′, 6′$_1$ = 2.5; 5′, 6′$_2$ = 5; 6′$_1$, 6′$_2$ = 12.