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STEROID GLYCOSIDES OF *Nicotiana tabacum* SEEDS
I. THE STRUCTURES OF NICOTIANOSIDES A, B, AND E

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Two steroid glycosides of the spirostan series — nicotianosides A and B — and one glycoside of the furostan series — nicotianoside E — have been isolated from the seeds of Nicotiana tabacum *L. Nicotianoside A is (25S)-5α-spirostan-3β-ol 3-O-β-D-glucopyranoside, nicotianoside B is (25S)-5β-spirostan-3β-ol 3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside], and nicotianoside E is (25S)-5α-furostan-3β,22α,26-triol 26-O-β-glucopyranoside 3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside].*

In the present paper we give a proof of the chemical structures of three new steroid glycosides that we have isolated from the seeds of *Nicotiana tabacum* L. By the repeated chromatographic separation of a methanolic extract of the seeds on a column of silica gel we isolated three individual steroid glycosides, which we have called nicotianosides A (**1**), B (**2**), and C (**3**). Compounds (**1**), and (**2**) gave a positive reaction with the Sannié reagent [1] and a negative one with the Ehrlich reagent [2], which showed their spirostanol nature. The IR spectra of (**1**) and (**2**) contained the absorption bands 895 < 915 that are characteristic for a spiroketal chain of the (25S)-series [3]. From its positive reactions with the Sannié and Ehrlich reagents, and also from its absorption in the IR spectrum, compound (**3**) was assigned to the steroid glycosides of the furostan series.

The complete acid hydrolysis of the nicotianosides led to the isolation in each case of a genin, which was identified from its physicochemical constants and its ^{13}C NMR spectrum as neotigogenin (**4**). But in view of the fact that compound (**3**) was assigned to glycosides of the furostan series, its native aglycon is (25S)-5α-furostan-3β,22α,26-triol.

By the PC and GLC of the aldononitrile acetate derivatives of the monosaccharides, in the oligosaccharide part of the glycosides we identified glucose as a component of compound (**1**), glucose and rhamnose in a ratio of 1:1 in (**2**), and glucose and rhamnose in a ratio of 2:1 in (**3**). The results obtained were confirmed by the ^{13}C NMR spectra of the glycosides: in the region of anomeric atoms the spectrum of (**1**) showed one signal (103.5 ppm), that of (**2**) showed signals at 102.8 and 100.3 ppm, and that of (**3**) signals at 105.2, 102.1, and 100 ppm (Table 1). The positions of attachment and the sizes of the oxide rings of the monosaccharides in each nicotianoside were determined by Hakomori methylation [4], followed by the methanolysis of the derivatives obtained. By GLC in the presence of authentic markers, for the permethylated (**1**) we identified methyl 2,3,4,6-tetra-O-Me-*D*-glucopyranoside (**5**); for (**2**) — methyl 2,3,4-tri-O-Me-*L*-rhamnoside (**6**) and methyl 3,4,6-tri-O-Me-*D*-glucopyranoside (**7**); and for (**3**) — (**5**), (**6**), and (**7**).

The sequence of attachment of the monosaccharide residues in the nicotianosides was determined with the aid of partial acid hydrolysis. In the case of (**2**), a progenin was obtained which broke down on hydrolysis to give neotigogenin and glucose. Consequently the glucose residue was attached directly to the aglycon. From its physical constants, this progenin was identical with nicotianoside A. When (**3**) was cleaved, two progenins were obtained. One of them was identical with nicotianoside A, and the other, containing neotigogenin, glucose, and rhamnose residues in a ratio of 1:1:1, with nicotianoside B.

When the ^{13}C NMR spectra of neotigogenin and nicotianoside B were compared, it was seen that in (**2**) the signal of the C-3 atom of the genin had shifted downfield by 6.7 ppm (Table 1), while the signals of the C-2 and C-4 atoms had shifted upfield. The chemical shifts of the other carbon atoms were unchanged. This is explained by the glycosylation effect arising on the attachment of a sugar residue to the hydroxy group at C-3 of the aglycon.

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TABLE 1. Chemical Shifts of the ^{13}C Carbon Atoms of Neotigigenin (**4**), Nicotianoside B (**2**) and Nicotianoside E (**3**) (δ, ppm, 0 — TMS, C_5D_5N)

C atom	Compound			C atom	Compound	
	4	2	3		2	3
1	37.0	37.4	37.3		β-*D*-Glc$_p$ at C-3	
2	31.5	30.0	30.0	1	100.3	100.0
3	71.3	78.0	77.5	2	78.7	78.9
4	38.1	34.6	35.9	3	78.5	78.0
5	44.6	44.8	44.8	4	71.6	71.4
6	28.6	29.1	29.1	5	76.8	76.6
7	32.2	32.6	32.3	6	61.3	61.1
8	35.0	35.4	35.3		α-*L*-Rha$_p$	
9	54.3	54.7	53.9	1	102.8	102.1
10	35.6	36.1	36.0	2	72.5	72.7
11	21.0	21.5	21.4	3	72.9	72.9
12	40.2	40.3	39.1	4	73.9	74.0
13	40.6	40.9	40.8	5	69.4	69.7
14	56.6	56.6	56.8	6	18.4	18.8
15	31.9	32.3	32.3		β-*D*-Glc$_p$ at C-26	
16	80.9	81.4	79.0	1		105.2
17	62.4	63.0	63.5	2		75.4
18	16.5	16.8	17.0	3		78.7
19	12.1	12.6	12.6	4		71.9
20	42.1	42.6	44.8	5		78.6
21	14.3	15.0	13.9	6		63.0
22	109.3	109.9	112.9			
23	27.0	27.7	34.6			
24	25.9	26.5	28.1			
25	26.2	26.3	37.2			
26	65.1	65.3	75.4			
O-CH_3		–	49.5			

In the low-field region of the ^{13}C NMR spectrum of nicotianoside E (**3**), as compared with that of (**2**), the signals of the C-23, C-24, C-25, and C-27 atoms had shifted downfield, which is characteristic for steroids of the furostan series, while a downfield shift of the signal of the C-26 atom from 65.3 to 75.4 ppm corresponded to a glycosylated form of a furostan [5]. From this followed the conclusion that the centers of glycosylation in compound (**3**) were C-3 and C-26 of the aglycon.

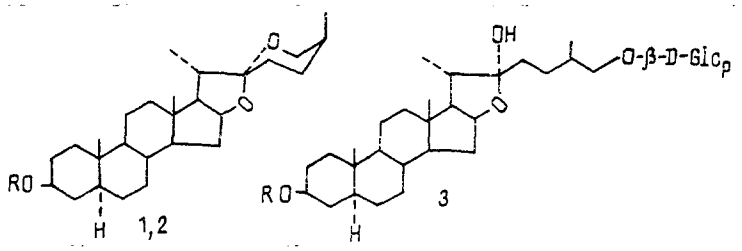
The assignment of the signals of the C-atoms of the rhamnose and glucose residues was made with the use of literature information for unsubstituted methyl α-*L*-rhamnopyranoside and methyl β-D-glucopyranoside [5] and for the chemical shifts of terminal rhamnose and glucose residues in various glycosides [6]. A comparison of the chemical shifts of glucose with its terminal analogues in related compounds showed substitution at the C-2 atom of the glucose residue in compounds (**2**) and (**3**) (downfield shifts from 75.4 to 78.7 ppm and from 75.4 to 78.9 ppm, respectively).

The ^1H and ^{13}C NMR spectra of compounds (**1**) and (**3**) showed the α-configuration of the anomeric center of the rhamnose residue and the β-configuration of the anomeric centers of the glucose residues (Tables 1 and 2). The nature of the splitting of the signals of the skeletal protons of the monosaccharide residues and the SSCCs confirmed their rhamno- and

TABLE 2. Chemical Shifts (ppm, 0 — TMS, C_5N_5N) and SSCCs (J, Hz) of the Protons of of Nicotianosides B (2) and E (3)

Protons of the aglycons and sugars (2)	δ, ppm., J, Hz,	Protons of the aglycons and sugars (3)	δ, ppm., J, Hz,
H-3	3.84 m	H-3	3.78 m
CH_3-18	0.74 s	CH_3-18	0.71 s
CH_3-19	0.78 s	CH_3-19	0.70 s
CH_3-21	1.07 d, $J_{20,21}$=7.0	CH_3-21	1.1 d, $J_{20,21}$=6.8
CH_3-27	1.02 d, $J_{25,27}$=7.0	CH_3-27	1.09 d, $J_{25,27}$=7.1
			β-*D*-Glucose at C-3
1	4.87 d, $J_{1,2}$=7.5	1	4.85 d, $J_{1,2}$=7.5
2	4.05 t, $J_{2,3}$=7.5	2	4.05 dd, $J_{2,3}$=7.5
3	4.09 dd, $J_{3,4}$=10.0	3	3.82 dd, $J_{3,4}$=9.8
4	4.42 t, $J_{4,5}$=10.0	4	4.17 dd, $J_{4,5}$=9.0
5	3.62 m	5	3.59 m
6a	3.98 dd	6a	4.0 dd
6b	4.18 dd	6b	3.89 dd
			α-*L*-Rhamnose
1	6.16 s	1	5.62 s
2	4.68 d, $J_{2,3}$=3.5	2	4.54 d, $J_{2,3}$=2.5
3	4.45 dd, $J_{3,4}$=9.5	3	4.38 dd, $J_{3,4}$=9.5
4	4.21 t, $J_{4,5}$=9.5	4	4.20 dd, $J_{4,5}$=9.0
5	4.78 dq	5	4.79 m
6	1.64 d	6	1.67 d, $J_{6,5}$=7.0
			β-*D*-Glucose at C-26
		1	4.7 d, $J_{1,2}$=75
		2	3.89 dd, $J_{2,3}$=8.0
		3	4.08 dd, $J_{2,3}$=8.0
		4	3.82 t
		5	4.43 m
		6a	4.23 dd
		6b	4.26 dd

gluco-configurations in the pyranose forms. On the basis of the facts given above, the following structures are proposed for nicotianosides A (1), B (2), and C (3):



1. R=β-*D*-Glc$_p$
2. R=α-*L*-Rhap$\xrightarrow{2}$β-*D*-Glc$_p$
3. R=α-*L*-Rha$_p\xrightarrow{2}$β-*D*-Glc$_p$

EXPERIMENTAL

For chromatography we used silica gel 100/160 μm and 5/40 μm + 13% of gypsum, FN-12 paper, and the solvent systems: 1) chloroform—methanol (9:1), 2) chloroform—methanol (4:1), 3) chloroform—methanol—water (65:30:10, lower layer), 4) benzene—ethanol (9:1), 5) benzene—diethyl ether (7:3), and 6) butanol—benzene—pyridine—water (5:1:3:3, upper layer). For revealing the spots we used the Sannié reagent (1% alcoholic solution of vanillin), the Ehrlich reagent (1% alcoholic solution of *p*-methylaminobenzaldehyde), a solution of aniline phthalate, and sulfuric acid.

GLC analysis was conducted on a Chrom-5 chromatograph with, for the sugar derivatives, a glass column 2.4 m long filled with 5% of XE-60 on Chromaton N-AW-HMDS. The carrier gas was helium. The temperature program for the chromatography of the aldononitrile acetate derivatives of the sugars was 180-230°C at 3°C per minute, while the temperature for the chromatography of the methylated nethyl glycosides was 140°C, with a rate of flow of the carrier gas of 45 ml/min.

Melting points were determined on a Boetius stage, ands specific rotations on a Zeiss polarimeter. IR spectra were taken on a Specord 71-IR spectrophotometer, mass spectra on a MKh 1303 instrument, PMR spectra on AM-300 and WM-250 instruments (Bruker) for solutions in deuteropyridine at 70°C, and ^{13}C NMR spectra on an AM-300 instrument under analogous conditions.

Isolation of the Steroid Glycosides. Air-dry tobacco seeds (0.5 kg) were ground, defatted with chloroform, and extracted with 70% methanol. The resulting extract was concentrated in vacuum and was then chromatographed repeatedly on silica gel, using solvent systems 1, 2, and 3, succssively. This led to the isolation of 650 mg of nicotianoside A (**1**), mp 276°C, $[\alpha]_D^{20}$ −65° (*c* 1.0; CH_3OH), 1450 mg of nicotianoside B (**2**), mp 241-242°C, $[\alpha]_D^{20}$ −56°C (*c* 1.0; CH_3OH), and 800 mg of nicotianoside E (**3**), mp 178°C $[\alpha]_D^{20}$ −78°C (*c* 1.0; CH_3OH).

Acid Hydrolysis of the Nicotianosides. Compounds (**1**), (**2**), and (**3**) (50 mg each) were hydrolyzed with 2.5% H_2SO_4 at 110°C for 8 h. In each case, the reaction mixture was diluted with water, and the aglycon liberated by the reaction was extracted with diethyl ether. The combined extrct was washed with water to neutrality, evaporated to a dry residue, and chromatographed in system 5. For each of the nicotianosides the aglycon was identified as neotigogenin, mp 202-203°C, $[\alpha]_D^{20}$ −75° (*c* 1.0; $CHCl_3$). M^+ 416. IR spectrum (cm^{-1}): 3360, 960, 920>900, 862, 835. Its ^{13}C NMR spectrum agreed with that for neotigogenin [7]. The paper chromatography in system 6 and GLC of the aldononitrile acetate derivatives of the sugars [8] revealed glucose in the hydrolyzate of (**1**), glucose and rhamnose in a ratio of 1:1 for (**2**), and glucose and rhamnose in ratio of 2:1 for (**3**).

Methylation of the Nicotianosides. Solutions of each glycoside (40 mg) in 10 ml of dimethylsulfenyl anion solution (prepared from 700 mg of NaH and 30 ml of DMSO) were stirred at 50°C in an atmosphere of argon for 1 h. Then CH_3I (1 ml) was added to each reaction mixture and it was left in the dark at room temperature for 12 h. After this, it was diluted with water and extracted with chloroform, and the extract was washed successively with with saturated $Na_2S_2O_3$ and with water, and was concentrated in vacuum. The methylation product was purified by chromatography on a column of silica gel in system **4**. Methanolysis of the permethylates obtained was conducted with 72% $HClO_4$ in methanol (1:10) at 100°C for 6 h. The hydrolyzates were neutralized with an anion-exchange resin, and evaporated. GLC in the presence of authentic markers revealed the methyl glycosides mentioned in the discussion.

Partial Hydrolysis of the Nicotianosides. Solutions of compounds (**2**) and (**3**) (200 mg in each case) in 30 ml of 1% H_2SO_4 in methanol were heated in the water bath for 1.5 h. The reaction mixtures were diluted with water and extracted with butanol. The butanolic extracts were evaporated and chromatographed on a column of SiO_2, with elution by systems 1 and 2 successively. Compound (**2**) yielded neotigogenin and (**1**) (87 mg), and compound (**3**) yielded (**1**) (46 mg) and (**2**) (59 mg).

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