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GLUCOSIDES OF IONONE-RELATED COMPOUNDS IN SEVERAL *NICOTIANA* SPECIES

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Key Word Index—*Nicotiana alata*; *N. repanda*; *N. undulata*; *N. accuminata*; *N. sylvestris*; *N. paniculata*; *N. tabacum*; Solanaceae; ionone-related compounds; glucosides.

Abstract—The β-glucosides of 3-oxo-α-ionol and 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol were isolated from fresh leaves of *Nicotiana rustica*. Two or more of the glucosides of 3-oxo-α-ionol, 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol, 3-hydroxy-β-damascone, blumenol A, 4-(3-hydroxybutylidene)-3,5,5-trimethyl-2-cyclohexenl-one and blumenol C were shown to be present and the amounts measured in *N. alata*, *N. repanda*, *N. rustica*, *N. undulata*, *N. accuminata*, *N. sylvestris* and *N. tabacum*. No glucosides were detected in *N. paniculata*.

INTRODUCTION

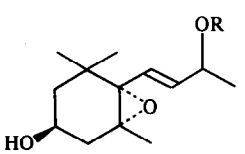
A large number of ionone-related compounds have been found in the essential oil of cured tobacco leaves. Most of these compounds have been thought to be generated by oxidative degradation of carotenoids during the curing and ageing process [1]. In the course of our studies on non-volatile constituents in tobacco, three β-glucosides of ionone-related compounds, blumenol A-β-glucoside, 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside and loliolide-β-glucoside were isolated from flue-cured tobacco leaves [2, 3]. These glucosides were present at the levels of *ca* 10 ppm. In this paper we report on the isolation of 3-oxo-α-ionyl-β-glucoside and 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside from fresh leaves of *Nicotiana rustica* and the types and amounts of the β-glucosides of ionone-related compounds in fresh leaves of several species of the genus *Nicotiana*.

RESULTS

Isolation of glucosides from N. rustica

A methanol extract of fresh green leaves of *N. rustica* was separated by high porous resin column chromatography into three fractions: water, 20% ethanol and ethanol. The main component of the water fraction was identical with authentic glucose (R_f on TLC). The 20% ethanol fraction was separated by Bio-gel P-2 column chromatography into six fractions (Fr. 1–6) from one (Fr. 5) of which glucoside **2** was isolated as a colourless oil by HPLC (μ-bondapak C_{18}).

The ^{1}H NMR spectrum of **2** was identical with authentic 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside obtained from flue-cured tobacco [3]. Enzymic hydrolysis of **2** with β-glucosidase liberated the aglycone (**1**) of **2**. The EIMS of the aglycone was identical with that of authentic 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol



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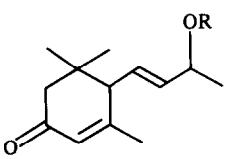
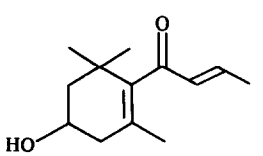
2 R = β–Glc



3 R = H
4 R = β–Glc



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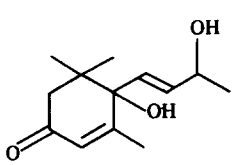

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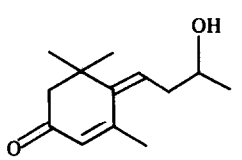

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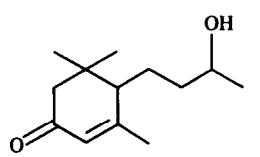

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obtained from the essential oil of tobacco. The structure of **2** was then confirmed to be 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside.

The ethanol fraction was acetylated (acetic anhydride–pyridine) and the acetates separated by silica gel column chromatography (hexane–ether). The acetate of glucoside **4** was isolated from the ether fraction by HPLC (Shodex GPC). The EIMS of **4** acetate had $[M]^+$ at m/z 538 and fragmentation ions at m/z 429, 331, 271, 191, 169, 109, 69 and 43. The ions at m/z 331 and 191 were thought to be due to the elimination of the hydroxy group from the glucose and aglycone moiety of **4** acetate respectively. The ^{1}H NMR spectrum showed the presence of *gem* dimethyl groups (δ0,97 and 1.03, each 3H, *s*), an olefinic methyl group (1.88, 3H, *s*), four acetyl groups (2.00, 2.02, 2.04, 2.07, each 3H, *s*), H-5 of the sugar moiety (3.69, 1H, *m*), an anomeric proton (4.57, 1H, *d*, J = 7.6 Hz), ring protons of the sugar moiety (4.17, 3H, *m* and 4.9–5.2, 3H, *m*) and olefinic protons (5.61, 2H, *m* and 5.89, 1H, *s*, *br*). These data suggested the structure of **4** acetate to be 3-oxo-α-ionyl-β-glucoside tetraacetate. **4** acetate was saponified (NH_3–MeOH) and hydrolysed by β-glucosidase to give the aglycone (**3**) of glucoside **4**. The EIMS of the aglycone was identical with authentic 3-oxo-α-ionol obtained from the essential oil of tobacco.

Contents of terpenoid glucosides

The types and amounts of glucosides of ionone-related compounds were determined in the leaves of eight species of the genus *Nicotiana*. Freeze-dried fresh leaves from two-month old plants of each species were washed with dichloromethane, and the residue suspended in boiling methanol to inactivate the native enzymes. The methanol extracts were dissolved in water and extracted with *n*-butanol. The *n*-butanol extract was washed with acid and base to give a glucoside-containing fraction, which was treated with β-glucosidase to liberate the aglycones. The aglycones were identified qualitatively and quantitatively by means of capillary GC/MS and GC. No aglycones were detected in the experiment performed in a similar manner without enzyme. The types and amounts of each aglycone are shown in Table 1. Six ionone-related compounds were identified from seven species of the genus *Nicotiana*. No aglycones were detected in *N. paniculata*.

Table 1. Distribution and amounts of compounds **1**, **3** and **5–8**

	Aglycone (μg/g dry wt.)					
Species	**1**	**3**	**5**	**6**	**7**	**8**
N. alata	1.19	0.39			*	*
N. repanda	1.04	0.01	*	0.44	1.16	
N. rustica	*	0.85				
N. undulata	1.15	*			1.62	1.24
N. accuminata	3.88	1.24			1.81	
N. sylvestris		*				
N. paniculata		——not detected——				
N. tabacum		0.47				0.23

1, 5,6-epoxy-5,6-dihydro-3-hydroxy-β-ionol; **3**, 3-oxo-α-ionol; **5**, 3-hydroxy-β-damascone; **6**, blumenol A; **7**, 4-(3-hydroxybutylidene)-3,5,5-trimethyl-2-cyclohexane-1-one; **8**, blumenol C. *, Less than 0.01 μg/g dry wt.

DISCUSSION

Among tobacco aroma constituents, ionone-related compounds are main components. These compounds have been thought to be derived from carotenoids during the curing and ageing process. There have been few reports about the occurrence of ionone-related compounds in fresh tobacco leaves [1]. But in this study, it was suggested that ionone-related compounds were present as glucosides in fresh tobacco leaves. On the other hand, recent studies on monoterpene alcohols [4, 5] confirmed that glucosides of these alcohols are present widely in plant species and that they are hydrolysed by β-glucosidase to liberate monoterpene alcohols as perfume. The results of this paper suggest that in tobacco leaves a part of ionone-related compounds is derived from glucosides by β-glucosidase during the ageing and curing process.

EXPERIMENTAL

Materials. Plants were grown in a greenhouse at 24°. After 2 months, fresh leaves were harvested, frozen immediately and lyophilized.

Instruments. GC (aglycone): FID, 50 m × 0.28 mm (i.d.) glass capillary column coated with OV-101; column temp., 100–240° at 2°/min; HPLC: Waters solvent delivery system M 6000 A constant flow pump and R 401 differential refractometer.

Isolation of glucoside containing fraction. Fresh leaves (30 g) were washed with CH_2Cl_2 (800 ml) and suspended in boiling MeOH (800 ml) for 15 min and kept overnight at room temp. The suspension was filtered and the residue re-extracted with MeOH (500 ml). The MeOH extracts were evaporated and then dissolved in H_2O (100 ml) and washed with Et_2O (50 ml × 2). The aq. layer was extracted with *n*-BuOH (100 ml), and the *n*-BuOH extract washed with 100 ml aq. 5% $KHPO_4$, H_2O, aq. 5% $NaHCO_3$, aq. satd NaCl and H_2O, and then evaporated to give a glucoside containing fraction.

Enzyme hydrolysis. Almond β-glucosidase (EC 3.2.1.21, Boehringer mannheim, 20 U/mg protein) had no activity for α-benzyl glucoside. Half of the glucoside-containing fraction was dissolved in Pi buffer (pH 5.38) and incubated with β-glucosidase (1 mg) at 38°. After 16 hr, the buffer soln was extracted with Et_2O (30 ml). The extract was dried ($MgSO_4$) and then evaporated to give an analytical sample. Another half of the glucoside-containing fraction was incubated without β-glucosidase under the same conditions and treated as above. Liberated aglycones were measured by capillary GC and GC/MS with 10 μl of 2,3,6-trimethylnaphthalene (1 mg/ml) as an internal standard.

Isolation of glucosides **2** *and* **4**. Fresh leaves of *N. rustica* (2 month old, 1169 g) were extracted with MeOH (2 l). The MeOH extracts (41 g) were separated by high porous resin column chromatography (DIAION HP-20) into three fractions: H_2O (34.9 g), 20% EtOH (3.53 g) and EtOH (3.28 g). The 20% EtOH fraction was further separated on Bio-gel P-2 into six fractions (Frs 1–6) which were monitored by the phenol–H_2SO_4 method [6]. Glucoside **2** was isolated from Fr. 5 (254 mg) by HPLC (μ-bondapak C_{18}). The EtOH fraction was treated with Ac_2O–pyridine to give acetate derivatives. **4** acetate was isolated from the Et_2O fraction by HPLC (Shodex GPC).

5,6-Epoxy-5,6-dihydro-3-hydroxy-β-ionyl-β-glucoside (**2**). ^{1}H NMR (100 MHz, C_5D_5N): δ1.11 (3H, *s*), 1.15 (3H, *s*), 1.26

(3H, *d*, J = 6 Hz), 1.5–2.6 (4H, *m*), 3.8–4.5 (6H, *m*), 4.87 (1H, *d*, J = 7.3 Hz).

Aglycone of **2**. EIMS 70 eV, *m/z*: 208, 152, 125, 123, 109, 107, 95, 82 and 55.

3-*Oxo-α-ionyl-β-glucoside tetraacetate*. ^{1}H NMR (100 MHz, $CDCl_3$): δ0.97 (3H, *s*), 1.03 (3H, *s*), 1.23 (3H, *d*, J = 5.2 Hz), 1.88 (3H, *s*), 2.00 (3H, *s*), 2.02 (3H, *s*), 2.04 (3H, *s*), 2.07 (3H, *s*), 3.69 (1H, *m*), 4.17 (3H, *m*), 4.57 (1H, *d*, J = 7.6 Hz), 4.9–5.2 (3H, *m*), 5.61 (2H, *m*), 5.89 (1H, *s*, *br*). EIMS 70 eV, *m/z*: 538, 429, 331, 271, 191, 169, 109, 69 and 43.

Aglycone of **4**. EIMS 70 eV, *m/z*: 152, 109, 108, 95, 91, 79, 77 and 43.

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