Rapid Paper

Isolation of a New Terpene Glucoside,
Loliolide- β -D-glucopyranoside
from Flue-cured Tobacco[†]Hisashi KODAMA, Takane FUJIMORI
and Kunio KATŌ*Central Research Institute,
The Japan Tobacco & Public Corporation,
6-2 Umegaoka, Midori-ku,
Yokohama 227, Japan*

Received November 6, 1981

A C_{11} -nor-carotenoid glucoside isolated from flue-cured tobacco was identified as loliolide- β -D-glucopyranoside from the spectral data and synthetic method.

A large number of the volatile flavor constituents of tobacco have been thought to be generated by oxidative degradation of terpenoids such as carotenoids and thunberganoids during maturing and post-harvest treatment. In contrast with many examinations of nor-carotenoids, very few reports on the glycosides of these compounds have been published. We have previously reported the isolation of a new C_{13} -nor-carotenoid glucoside, 5,6-epoxy-5,6-dihydro-3-hydroxy- β -ionyl- β -D-glucopyranoside, from the non-volatile constituents of flue-cured tobacco.¹⁾ In this paper, we report the isolation of a new C_{11} -nor-carotenoid glucoside and its identification as loliolide- β -D-glucopyranoside.

The isolation procedure for glycoside I was illustrated in Fig. 1. By the isolation procedure described in the previous paper,¹⁾ the MeOH No. 2 fraction (570 mg) was obtained from flue-cured tobacco (3 kg).

Further, the MeOH No. 2 fraction was separated by column chromatography on Biogel P-2. The effluent monitored by the phenol-sulfuric acid method²⁾ showed five peaks (F1~F5), and F3 was successively separated twice by high performance liquid chro-

FIG. 1. Isolation Procedure of Glycoside I.

FIG. 2. High Performance Liquid Chromatogram of F3.

FIG. 3.

[†] Non-volatile Constituents in Tobacco. Part II. For Part I, see ref. 1.

matography (HPLC). Glycoside I, corresponding to peak A, was obtained as a colorless oil as shown in Fig. 2. The molecular weight of glycoside I was determined to be 358 by FD mass spectrometry. The FD mass spectrum of glycoside I showed the cation clusters of the molecule, and $[M + Na]^+$ ion at m/z 381 as the base peak and an $[M + ^{39}K]^+$ ion at m/z 397. The 1H -NMR spectrum of glycoside I showed the presence of *gem*-dimethyls (δ 1.09, 1.41), one CH_3COR group (δ 1.95), one olefinic proton (δ 5.81), one anomeric proton (δ 4.91, d, $J=7.1$ Hz) and ring protons of the sugar moiety (δ 3.8~4.4). The infrared spectrum of glycoside I indicated the hydroxyl group (3350 cm^{-1}) and α,β -unsaturated lactone (1725 cm^{-1}). The enzymatic hydrolysis of glycoside I with β -glucosidase afforded an aglycone. The aglycone was identified with synthetic loliolide by means of GC-MS. From the above observations, glycoside I was presumed to be loliolide- β -D-glucopyranoside 1.

To confirm the above structural elucidation, loliolide- β -D-glucopyranoside was prepared by the method of Koenigs-Knorr.³⁾ Loliolide was condensed with acetobromoglucose to give loliolide-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside 2. In this reaction the α -anomer was not obtained. Loliolide-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside was saponified with methanolic ammonia to give loliolide- β -D-glucopyranoside. The 1H -NMR and IR spectra of the prepared loliolide- β -D-glucopyranoside were identical with those of glycoside I obtained from flue-cured tobacco. The new terpene glycoside I was thus inferred to be loliolide- β -D-glucopyranoside. The aglycone of this glycoside, loliolide, had already been found in volatile constituents of tobacco.⁴⁾ The absolute configuration of the aglycone, however, could not be determined owing to the small amount of the sample. This is the first example of the occurrence of 1 in nature. Up to the present, there has been no clear explanation for the formation of tobacco nor-carotenoids. Nor-carotenoid glucoside is considered to be a precursor of the nor-carotenoid volatile flavor constituents.

EXPERIMENTAL

Concentration was performed under reduced pressure at a bath temperature not exceeding 45°C .

Enzymatic hydrolysis. β -Glucosidase (from sweet almonds; Boehringer Mannheim) had no activity for α -benzyl glucoside. Glycoside I was suspended in potassium phosphate buffer (pH 5.25) and β -glucosidase was added at 38°C . After 24 hr aglycone was extracted from the solution with chloroform.

High performance liquid chromatography (HPLC). HPLC was performed with a Waters solvent delivery system M 6000A constant flow pump and for monitoring the column effluent a Waters R 401 differential refractometer was used.

Instrumental analyses. FD and EI mass spectra were obtained with a Hitachi M-80. Infrared spectra were obtained with a Jasco A-3. Specific rotation was measured with a Jasco DIP-181. Proton NMR spectra were obtained with a JEOL FX-100 Fourier transfer NMR spectrometer. The chemical shifts are expressed as ppm down field from Me_4Si as an internal standard.

Isolation of glycoside I. The isolation procedure for the MeOH No. 2 fraction was described in the previous report.¹⁾ On top of a column ($126 \times 2\text{ cm i.d.}$), packed with a Biogel P-2, MeOH No. 2 fraction was placed and the column was eluted with water. By the phenol- H_2SO_4 method, the MeOH No. 2 fraction was separated into five sugar containing fractions.

Fraction 3 among the five fractions was successively separated twice by HPLC. Glycoside I was obtained as a colorless syrup.

$[\alpha]_D = -21.25$ ($c=0.08$: EtOH) 1H -NMR δ (pyridine- d_5): 1.09 (3H, s), 1.41 (3H, s), 1.95 (3H, s), 2.15 (1H, broad d, $J=14.1$ Hz), 2.83 (1H, broad d, $J=14.1$ Hz), 3.8~4.4 (7H, m), 4.91 (1H, d, $J=7.1$ Hz), 5.81 (1H, s), IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3350, 1725 FD mass: m/z 359, 381, 397.

*Lololide-2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranoside 2.* Loliolide (100 mg) and α -acetobromoglucose (200 mg) were dissolved in 50 ml of CH_2Cl_2 and the solution was stirred with freshly prepared Ag_2CO_3 (100 mg) at room temperature for 1 day. The reaction mixture was filtered and then evaporated under reduced pressure. The residue was separated on a silicic acid (6 g) column. Elution with 40% EtOAc/hexane successively on HPLC (Shodex GPC H202: CHCl_3) yielded 2 as white crystals (10.6 mg, 4%).

1H -NMR δ (CDCl_3): 1.24 (3H, s), 1.35 (3H, s), 1.60 (3H, s), 2.02 (12H, s), 3.7 (1H, m), 4.2 (3H, m), 4.58 (1H, d, $J=7.4$ Hz), 4.90~5.24 (3H, m), 5.67 (1H, s), IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1760, 1730, 1380, 1365. Mass (70 eV): m/z (%): 43 (97), 81 (48), 98 (46), 109 (69), 115 (42), 135 (56), 169 (48), 179 (100), 225 (42), 331 (5), 406 (0.3), 411 (0.2), 467 (0.2), 527

(0.1).

loliolide.

Saponification of loliolide-2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside 2. With saturated NH_3 in MeOH, 10 mg of **2** was saponified to give loliolide- β -D-glucopyranoside in a quantitative yield.

$^1\text{H-NMR}$ δ (pyridine- d_5): 1.06 (3H, s), 1.40 (3H, s), 1.94 (3H, s), 2.14 (1H, broad d, $J=14.1$ Hz), 3.8~4.5 (7H, m), 4.90 (1H, d, $J=7.2$ Hz), 5.80 (1H, s) IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3350, 1725.

Acknowledgment. We thank Dr. A. Satō and Dr. T. Satō of Takasago Perfumery Co., Ltd. for the synthesis of

REFERENCES

- 1) H. Kodama, T. Fujimori and K. Katō, *Agric. Biol. Chem.*, **45**, 941 (1981).
- 2) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
- 3) W. Koenigs and E. Knorr, *Chem. Ber.*, **34**, 957 (1901).
- 4) D. Behr, I. Walberg, T. Nishida and C. R. Enzell, *Acta Chem. Scand.*, **B33**, 701 (1979).