Pergamon

0031-9422(94)E0190-4

Phytochemistry, Vol 36, No. 6, pp 1435–1437, 1994

0031-9422/94 $7.00+0.00

LINALYL β-D-GLUCOPYRANOSIDE AND ITS 6′-*O*-MALONATE AS AROMA PRECURSORS FROM *JASMINUM SAMBAC*

JAE-HAK MOON, NAOHARU WATANABE,*† KANZO SAKATA,† JUNJI INAGAKI,† AKIHITO YAGI,† KAZUO INA† and SHAOJUN LUO‡

United Graduate School of Agricultural Sciences, Gifu University (Shizuoka University, 836 Ohya, Shizuoka 422, Japan); †Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422, Japan; ‡Hangzhou Tea Processing Research Institute, Ministry of Commerce, 46-2 Kaixuan Road, Hangzhou, P. R. China

(*Received in revised form* 21 *February* 1994)

Key Word Index—*Jasminum sambac*; Oleaceae; linalyl β-D-glucopyranoside; linalyl 6′-*O*-malonyl-β-D-glucopyranoside; monoterpene alcohol glycoside; aroma precursors; linalool.

Abstract—Linalyl β-D-glucopyranoside **1** and its 6′-*O*-malonate **2** were isolated as aroma precursors of linalool from flower buds of *Jasminum sambac* guided by enzymatic hydrolysis followed by GC and GC-MS analyses.

INTRODUCTION

In China several kinds of scented tea are produced by absorbing the fragrance of flowers on to parched green tea leaves. Jasmine tea, made by scenting parched green tea with floral fragrance of jasmine (*Jasminum sambac* Ait), is the most famous. The scenting process, in which moistened green tea leaves and just opening jasmine flowers are mixed a couple of times, is very important and requires intensive labour.

Recently, Luo *et al.* [1] reported that the aroma of scented tea is derived mainly from the flower used. Some monoterpene alcohols, which are important constituents of tea aroma, have been shown to be formed mainly from their glycosidic precursors by enzymatic hydrolysis during tea processing [1–3]. Quite recently, we have isolated new geranyl and linalyl disaccharides as aroma precursors from oolong tea leaves (*Camellia sinensis* var. *sinensis* cv Suixian) [4–6]. We also have confirmed that flower fragrance formation from glycosidic precursors by endogenous enzymatic hydrolysis occurs just before flower opening [7].

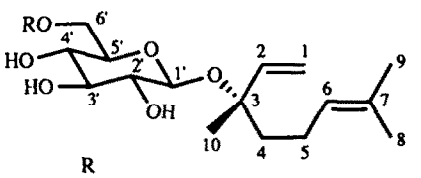
Several aldehydes, alcohols, etc. were reported to be important aroma constituents of jasmine flower by Kobayashi *et al.* [8]. They also reported that linalool is one of the most important determinants of the quality of jasmine tea [9]. Therefore, we focused our research interest on the mechanism of aroma formation in jasmine flowers. In this study, we attempted to isolate aroma precursors of linalool from jasmine flower buds.

RESULTS AND DISCUSSION

Flower buds just before anthesis were extracted with 80% methanol under ice-cooling. The extract was concentrated and chromatographed on an Amberlite XAD-2 column (H_2O–MeOH). Isolation of linalool glycosides was guided by detection of linalool on GC or GC-MS after enzymic hydrolysis of glycoside-containing fractions. Linalyl glycosides were found in the 60 and 100% MeOH fractions, which were hydrolysed with either an acetone powder prepared from jasmine flowers or naringinase. As naringinase showed higher activity than the acetone powder, naringinase was used for monitoring the purification steps.

The glycosidic fractions were fractionated on a Sephadex LH-20 column (50% MeOH) to afford a monoterpene alcohol glycoside fraction, which was further purified on an ODS column (40–100% MeOH, stepwise elution). Linalyl glycosides were separately eluted in 40 and 80% MeOH fractions. HPLC of each fraction on an ODS column (20–60% MeCN, gradient elution) gave two linalyl glycoside fractions.

The less polar fraction (*ca* 38% MeCN) was subjected to HPLC (×2) on an ODS column (gradient elution of



R

1 H

2 –CO–CH_2–COONa

1″ 2″ 3″

*Author to whom correspondence should be addressed.

20–50% MeCN and then 30–80% MeOH) to give compound **1** (*ca* 2.5 mg from 404 g fr. wt of flower buds).

The molecular formula of **1**, $C_{16}H_{28}O_6$, was determined by HRFAB-mass spectrometry (*m/z* 339.1782; −0.2 mmu for $C_{16}H_{28}O_6Na$). A sequential *trans*-1,2-diaxial relationship of H-1′–H-5′ ($J = 6.0$–8.0 Hz) suggested that **1** was a linalyl glucoside. Its ^{13}C NMR spectrum (Table 1) showed it to be the known compound 3(*S*)-linalyl-β-D-glucopyranoside [10]. The diastereomeric (*R*) and (*S*)-linalyl β-D-glucopyranosides are easily distinguished since they show different ^{13}C NMR chemical shifts [11].

The polar linalyl glycoside fraction (*ca* 26% MeCN) was subjected to HPLC on an ODS column (gradient elution of 10–30% MeCN) to give the other linalyl glycoside (**2**, 3.1 mg from 550 g fr. wt of flower buds). In the ^1H NMR spectrum (D_2O) of **2** the presence of an anomeric proton signal at δ4.53 (*d*, $J = 7.8$ Hz) clearly indicated that the monosaccharide moiety must be in the pyranoside form, and the signal at δ3.35 (2H, *s*) suggested a malonyl moiety. The ^{13}C NMR spectrum (D_2O; Table 1) of **2** showed 20 carbon signals including 16 carbon signals due to a linalyl glycoside and four signals [δ47.2, 173.8, 176.6, and 188.6 (with a half intensity of the δ176.6)] of the mixture of a salt and a free form of malonate. The assignment was deduced by comparison of the spectral data with those of a linalyl glycoside (**1**) and a glycoside linked with a malonate moiety [12]. The downfield shift of H-6′a and H-6′b of **2** by 0.4 and 0.75 ppm from H-6′a and H-6′b of **1**, respectively, showed that a malonyl moiety was connected to OH-6′. The molecular formula of **2**, $C_{19}H_{29}O_9Na$, as determined by HRFAB-mass spectrometry (*m/z* 425.1777; −1.0 mmu for $C_{19}H_{30}O_9Na$), supports the structure.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (67.8 MHz)

C	**1** (pyridine-d_5)	**2** (D_2O)
1	113.9	118.6
2	144.5	114.3
3	79.9	84.4
4	40.8	41.4
5	23.0	25.1
6	125.5	127.3
7	131.0	136.3
8	25.7	27.6
9	24.1	19.8
10	17.7	25.1
1′	99.4	99.9
2′	75.2	75.8
3′	78.8	78.5
4′	71.8	72.6
5′	78.1	75.9
6′	62.9	66.7
1″		173.8
2″		47.2
3″		176.6
3″		188.6

A methanolic solution of **2** (1.5 mg) was passed through a small column of CM-Toyopearl 650 M (H^+) to obtain the free acid, which was treated with freshly prepared ethereal diazomethane to quantitatively yield a less polar compound. The ^1H NMR (D_2O) spectrum of which showed disappearance of the characteristic malonate signal (δ3.35, 2H, *s*) and perfect coincidence with that of linalyl glucoside (**1**). The structure was confirmed by FAB-mass spectral analysis (*m/z* 317 $[M+H]^+$; *m/z* 315 $[M-H]^-$). This facile elimination of the malonyl moiety can be rationalized as being initiated by the reaction of excess diazomethane on an active methylene proton of the malonyl group. On the basis of the above, **2** was concluded to be linalyl 6′-*O*-malonyl-β-D-glucoside.

Both compounds **1** and **2** liberated linalool when treated with an acetone powder prepared from either jasmine flowers or tea leaves. Linalool is an important aroma compound in jasmine tea as well as various kinds of flowers, tea and fruits and several linalyl glycosides have been isolated as aroma precursors [4–6, 13–15]. This is, to our knowledge, the first example of a glycosidic aroma precursor of linalool, in which a malonyl group is connected to its sugar moiety.

EXPERIMENTAL

Enzymatic hydrolysis. A mixture of an aroma precursor sample equivalent to 2 g of fresh jasmine flower buds and naringinase in 10 ml of 0.1 M citrate buffer (pH 5) was kept at 30° for 12 hr. The reaction mixture was subjected to conventional work-up [4, 7] to give an aroma concentrate for GC (PEG-20M fused silica capillary column, 25 m × 0.25 mm i.d.) and GC-MS (EI 70 eV) analyses [4, 7].

Isolation of compounds **1** *and* **2**. Isolation was guided by the above enzymatic hydrolysis followed by GC and GC-MS analyses. Jasmine flower (*Jasminum sambac* Ait.) buds before flower opening (600 g, harvested in the morning from 11–15 August 1991, at Xiaoshan, Zhejiang, China) were extracted with 80% MeOH (800 ml × 3) under ice-cooling immediately after plucking. Combined extracts were concd *in vacuo*. The resulting aq. soln was chromatographed on an Ambelite XAD-2 column (φ5.5 × 33 cm; H_2O–MeOH, stepwise). The 60 and 100% MeOH frs were then subjected to Sephadex LH-20 CC (φ4.2 × 81 cm; 50% MeOH) to afford a monoterpene alcohol glycoside fraction (0.8 to 1.0 bed volume). Each glycosidic fraction was purified on an ODS column with stepwise elution of 40–100% MeOH. One linalyl glycoside was eluted in the 40 and 60% MeOH fraction and the other in the 100% MeOH fraction. Repeated HPLC of the less polar fraction on an ODS column (YMC Packed D-ODS-5, φ 2.0 × 25 cm; gradient elution of 20–60% MeCN, and then 30–80% MeOH) using a UV detector (210 nm) gave compound **1**: 2.5 mg; $[\alpha]_D^{22}$ −160° (MeOH; *c* 4.0); HRFAB-MS (Pos., NOBA) *m/z*: 339.1782 (−0.2 mmu for $C_{16}H_{28}O_6Na$); ^1H NMR (400 MHz, D_2O): δ1.35, 1.62, 1.69 (3H each, *s*), 1.60–1.75 (H_2-4, *br*), 1.96–2.10 (H_2-5, *m*), 3.22 (H-2′, *t*, $J = 8.0$, 5.8 Hz), 3.36 (H-3′, H-4′, *m*), 3.46 (H-5, *m*), 3.67 (H-6′a, *br d*, $J = 12$ Hz), 3.85

(H-6'b, *br d*, $J = 12$ Hz), 4.05 (H-1', *d*, $J = 8.0$ Hz), 5.22 (H-6 *br t*, $J = 6.6$ Hz), 5.26 (H-1a, *d*, $J = 12.0$ Hz), 5.29 (H-1b, *d*, $J = 17.6$ Hz), 6.00 (H-2 *dd*, $J = 11.4$, 18.2 Hz); ^{13}C NMR: Table 1.

The polar glycosidic fraction was purified by HPLC (gradient elution of 10–30% MeCN) on an ODS column to give compound **2**: HRFAB-MS (Pos., NOBA) *m/z*: 425.1777 (−1.0 mmu for $C_{19}H_{30}O_9Na$); ^{1}H NMR (270 MHz, D_2O): δ 1.34, 1.62, 1.69 (3H each, *s*), 1.60–1.70 (H_2-4, *br*), 1.90–2.10 (H_2-5, *br*), 3.22 (H-2', *t*, $J = 7.8$ Hz), 3.35 (2H-2'', *s*), 3.45 (H-4', *t*, $J = 7.2$ Hz), 3.48 (H-3', *t*, $J = 8.9$ Hz), 3.59 (H-5', *m*), 4.25 (H-6'a, *dd*, $J = 12.2$, 5.4 Hz), 4.43 (H-6'b, *d*, $J = 12.7$ Hz), 4.53 (H-1', *d*, $J = 7.8$ Hz), 5.27 (H-1b, *d*, J, = 10.6 Hz), 5.21 (H-6, *br t*, $J = 6.5$ Hz), 5.29 (H-1a, *d*, $J = 17.8$ Hz), 5.95 (H-2, *dd*, $J = 17.8$, 10.6 Hz); ^{13}C NMR: Table 1.

Diazomethane treatment of compound **2**. A methanolic soln (1 ml) of **2** (1.5 mg) was passed through a column (ϕ 0.5 × 2.5 cm) of TSK gel CM Toyopearl 650M (H^+) to obtain the free acid. Excess ethereal CH_2N_2 was added to the effluent from the column. The reaction mixture was evapd to dryness and purified by HPLC (ODS, gradient elution of 10–30% MeCN) to give a linalyl glycoside whose spectral data [^{1}H NMR (270 MHz, D_2O) and FAB-MS] were completely identical with those of **1**.

REFERENCES

1. Luo, S., Guo, W. and Fu, H. (1991) *Proceedings of International Symposium on Tea Science*, p. 57. Shizuoka, Japan.
2. Yano, M., Okada, K., Kubota, K. and Kobayashi, A. (1990) *Agric. Biol. Chem.* **54**, 1023.
3. Yano, M., Joki, Y., Mutoh, H., Kubota, K. and Kobayashi, A. (1991) *Agric. Biol. Chem.* **55**, 1205.
4. Guo, W., Sakata, K., Watanabe, N., Nakajima, R., Yagi, A., Ina, K. and Luo, S. (1993) *Phytochemistry* **33**, 1373.
5. Guo, W., Sakata, K., Hosoi, R., Watanabe, N., Yagi, A., Ina, K. and Luo, S. (1993) *Proceedings of the 37th Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics*, p. 386. Okinawa, Japan.
6. Guo, W., Sakata, K., Watanabe, N., Yagi, A., Ina, K. and Luo, S. (1994) *Biosci. Biotech. Biochem.* **34** (in press).
7. Watanabe, N., Watanabe, S., Nakajima, R., Moon, J., Shimokihara, K., Inagaki, J., Etoh, H., Asai, T., Sakata, K. and Ina, K. (1993) *Biosci. Biotech. Biochem.* **57**, 1101.
8. Kobayashi, A., Kubota, K., Anzai, Y., Cheng, M. and Yamanishi, T. (1985) *Proceedings of the 29th Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics*, p. 129. Mie, Japan.
9. Yamanishi, T., Luo, S., Guo, W. and Kobayashi, A. (1987) *Proceedings of the 5th International Flavor Conference of Frontiers of Flavor*, p. 181. Chalkidiki, Greece.
10. Tanaka, N., Sakai, H., Murakami, T., Saiki, Y., Chen, C. and Iitaka, Y. (1986) *Chem. Pharm. Bull.* **34**, 1015.
11. Murai, F. and Tagawa, M. (1990) *Proceedings of the 34th Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics*. p. 264. Kagawa, Japan.
12. Fenny, P., Sachdev, K., Rosenberry, L. and Carter, M. (1988) *Phytochemisty* **27**, 3439.
13. Williams, P., Strauss, C., Wilson, B. and Massy-Westropp, R. (1982) *Phytochemistry* **21**, 2013.
14. Pabst, A., Barron, D., Semon, E. and Schreier, P. (1991) *Tetrahedron Letters* **32**, 4885.
15. Duque, C., Wintoch, H., Suarez, M. and Schreier, P. (1993) *Progress in Flavour Precursor Studies*, p. 279. Allured, Carol Stream.