

ISOLATION OF SECOIRIDOID ARTIFACTS
FROM *LONICERA JAPONICA*

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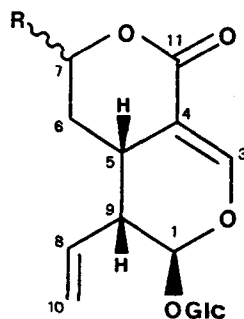
ABSTRACT.—Two artifactual secoiridoid glucosides, 7-*O*-butylsecologanic acid [**5**] and secologanin dibutylacetal [**6**], have been isolated from a butanol extract of *Lonicera japonica*, along with loganin, sweroside, and secologanin [**4**]. Evidence is provided that shows acetalic iridoids can be easily formed by reaction of the aldehydic function with alcoholic solvents.

Lonicera japonica Thunb. (Caprifoliaceae) is a herbaceous climber of oriental origin that is widely cultivated for ornamental purposes. The occurrence of three secoiridoid glucosides, namely, vogeloside [**1**], *epi*-vogeloside [**2**], and secologanin dimethylacetal [**3**], together with the iridoid glucoside loganin, has been reported (1). Among these compounds, **2** and **3** were isolated for the first time from a natural source (1). We now report the results of a reinvestigation of the iridoid constituents of this plant.

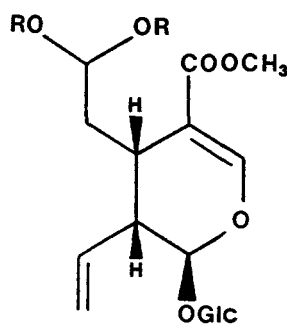
An EtOH extract of the aerial parts of *L. japonica* was concentrated *in vacuo*. After the subsequent addition of H₂O, the resulting solution was extracted, in turn, with EtOAc and *n*-BuOH. Separation by cc of the *n*-BuOH fraction over SiO₂ in CHCl₃-MeOH (8:2), led to the isolation of five pure compounds, namely, loganin, secologanin [**4**], sweroside, and two secoiridoids of novel structure, 7-*O*-butylsecologanic acid [**5**] and secologanin dibutylacetal [**6**]. Neither of the two vogeloside epimers (**1** and **2**), nor the secologanin dimethylacetal [**3**] was recovered. Due to the extraction procedure used, the differences from the previous investigation (1) can be considered a result of the formation of artifacts, by reaction of secologanin [**4**] or secologanic acid [**7**] with *n*-BuOH. To confirm this, the cc separation, on SiO₂ in CHCl₃-MeOH (9:1), of the EtOAc fraction afforded pure secologanin [**4**], together with a small amount of its dimethylacetal [**3**]. The latter compound (which was absent in the extract by tlc analysis) was

apparently formed by reaction between the aldehyde function of secologanin [**4**] and the alcoholic solvent (in this case the MeOH of the elution mixture).

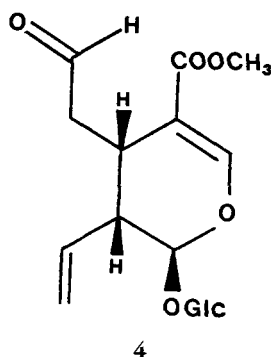
Further evidence for artifact formation was obtained by comparing two ¹H-nmr spectra of a CD₃OD solution of secologanin [**4**] (0.1%). After recording the first spectrum, the solution was allowed to stand for about ten days at room temperature, and the second ¹H-nmr spectrum showed, in addition to the expected signals of secologanin [**4**], resonances due to a mixture of its mono- and dimethylacetal derivatives. In particular, the transformation was evident in terms of the decrease of intensity of the singlet at δ 9.61 attributable to the aldehydic proton of secologanin [**4**], and in the appearance of a sharp multiplet at δ 4.55, due to the overlapping of the hemiacetal and acetal protons at C-7 of the secologanin derivatives. Furthermore, it



- 1** R=α-OMe
2 R=β-OMe
5 R=OBu
7 R=OH



3 R = Me
6 R = Bu



4

was possible to observe the occurrence of a small amount of secologanic acid [7] in the EtOH extract of the plant by tlc comparison with an authentic sample.

Therefore, on the basis of the aforementioned evidence, it appears that compounds like 1–3, reported previously in various papers as natural substances (1–3), as well as 5 and 6, can be formed easily as artifacts by the reaction of secologanin [4] or secologanic acid [7] with alcoholic solvents.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—¹H- and ¹³C-nmr spectra were recorded, at 500 and 125 MHz, respectively, on a Bruker AM 500 spectrometer, with CD₃OD used as solvent and TMS as internal standard. Ms were obtained on a VG-Prospec-Autospec Fisons instrument (fabms matrix thioglycerol).

PLANT MATERIAL.—*Lonicera japonica* Thunb. collected at the Botanical Garden of the Dipartimento di Biologia Vegetale, Università

“La Sapienza” di Roma. Identification was confirmed by Prof. B. Anzalone; the herbarium specimens are deposited at the Erbario del Dipartimento di Biologia Vegetale (RO).

EXTRACTION AND ISOLATION.—Fresh aerial parts (leaves and branches, 600 g) of the plant material were extracted exhaustively in 95% EtOH at room temperature. The extract was concentrated *in vacuo* and the residue subjected to partition between H₂O and EtOAc, with the aqueous layer re-extracted with *n*-BuOH. The *n*-BuOH extract was then subjected to cc on SiO₂ in CHCl₃-MeOH (8:2), yielding compounds 4–6. The cc separation of the EtOAc fraction was performed on SiO₂ in CHCl₃-MeOH (9:1) to afford compounds 3 and 4. The known substances, 1–4, were identified by comparison of their spectroscopic data with those reported in the literature (4). The occurrence of a small amount of secologanic acid [7] in the EtOH extract was observed by tlc comparison with an authentic sample obtained by alkaline hydrolysis of secologanin.

7-O-Butylsecologanic acid [5].—Amorphous powder; [α]_D²⁰ −21.4° (c=1, MeOH); uv (MeOH) λ max (log ε) 241 (3.97) nm; ir (KBr) ν max 3400, 1685, 1620 cm⁻¹; ¹H nmr δ 7.59 (1H, d, J=1.0 Hz, H-3), 5.55 (1H, br s, H-8), 5.48 (1H, d, J=8.2 Hz, H-1), 5.40 (1H, dd, J=9.0 and 1.5 Hz, H-7), 5.32–5.23 (2H, m, H₂-10), 4.69 (1H, d, J=7.7 Hz, H-1'), 3.94 (1H, m, H-1''a), 3.88 (1H, dd, J=12.0 and 1.5 Hz, H-6'a), 3.68 (1H, dd, J=12.0 and 4.8 Hz, H-6'b), 3.49 (1H, m, H-1''b), 3.40–3.28 (3H, m, H-3', H-4', H-5'), 3.20 (1H, t, J=7.7 Hz, H-2'), 3.10 (1H, m, H-5), 2.69 (1H, m, H-9), 1.98 (1H, dd, J=9.0 and 2.8 Hz, H-6a), 1.58 (2H, m, H-2''), 1.47 (1H, m, H-6b), 1.39 (2H, m, H-3''), 0.92 (3H, t, J=6.6 Hz, H₃-4''); ¹³C nmr (CD₃OD) δ 166.5 (C-11), 152.5 (C-3), 131.4 (C-8), 120.9 (C-10), 104.1 (C-4), 102.8 (C-7), 98.1 (C-1'), 96.7 (C-1), 76.1 (C-5'), 75.9 (C-3'), 72.9 (C-2'), 69.6 (C-4'), 69.2 (C-1''), 61.2 (C-6'), 42.1 (C-9), 30.5 (C-6), 31.5 (C-2''), 23.9 (C-5), 19.1 (C-3''), 13.8 (C-4''); fabms (positive-ion) m/z 431 [M+H]⁺, 374, 267.

Secologanin dibutylacetal [6].—Amorphous powder; [α]_D²⁰ −14.5° (c=0.9, MeOH); uv (MeOH) λ max (log ε) 236 (3.99) nm; ir (KBr) ν max 3400, 1700, 1620 cm⁻¹; ¹H nmr δ 7.37 (1H, s, H-3), 5.66 (1H, dd, J=17.2 and 10.3 Hz, H-8), 5.42 (1H, d, J=8.3 Hz, H-1), 5.28–5.17 (2H, m, H₂-10), 4.61 (1H, d, J=7.7 Hz, H-1'), 4.55 (1H, br s, H-7), 3.84 (1H, dd, J=12.0 and 1.5 Hz, H-6'a), 3.62 (3H, s, OMe), 3.60 (1H, dd, J=12.0 and 4.8 Hz, H-6'b), 3.52 (2H, m, 2×H-1'a), 3.38 (2H, m, 2×H-1'b), 3.35–3.21 (3H, m, H-3', H-4', H-5'), 3.12 (1H, t, J=7.7 Hz, H-2'), 2.89 (1H, m, H-5), 2.61 (1H, m, H-9), 2.04 (1H, dd, J=13.6 and

6.6 Hz, H-6a), 1.58 (1H, dd, $J=13.6$ and 4.7 Hz, H-6b), 1.48 (4H, m, $2\times\text{H-2}''$), 1.33 (4H, m, $2\times\text{H-3}''$), 0.90 (6H, t, $J=6.6$ Hz, $2\times\text{H-4}''$); ^{13}C nmr δ 169.1 (C-11), 153.1 (C-3), 135.7 (C-8), 119.8 (C-10), 111.7 (C-4), 103.0 (C-7), 100.0 (C-1'), 97.8 (C-1), 78.3 (C-5'), 78.0 (C-3'), 74.6 (C-2'), 71.5 (C-4'), 67.4, 66.1 (C-1''), 62.7 (C-6'), 51.7 (OMe), 45.3 (C-9), 34.0 (C-6), 33.1 (C-2''), 29.1 (C-5), 20.5, 20.4 (C-3''), 14.3, 14.2 (C-4''); fabms (positive-ion) m/z 519 $[\text{M}+\text{H}]^+$, 389, 357, 225.

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