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IRIDOIDS OF *RAUWOLFIA GRANDIFLORA**

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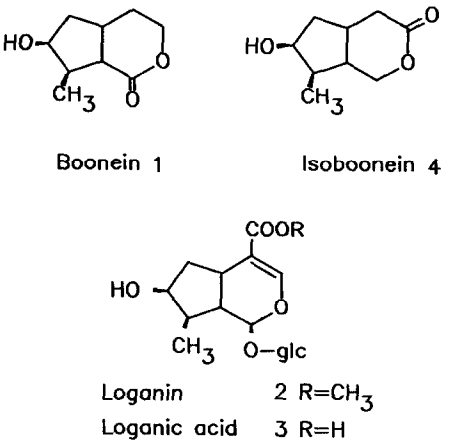
Key Word Index—*Rauwolfia grandiflora*; Apocynaceae; iridoid; loganin; loganic acid; boonein; isoboonein; monoterpene lactone.

Abstract—From the bark of *Rauwolfia grandiflora* a new monoterpenoid δ-lactone, isoboonein, was isolated together with boonein, loganin and loganic acid. The structure of isoboonein, established by spectroscopical methods, was confirmed by partial synthesis from loganic acid.

INTRODUCTION

Like some other members of the Apocynaceae, the genus *Rauwolfia* is characterized by the presence of indole alkaloids, e.g. reserpine [2], derived by the well-known tryptamine-loganin/secologanin biosynthetic pathway [3, 4]. The oxidoreductive rearrangment of the iridoidial precursor can give rise to irido-δ-lactones of either the nepetalactone-type or the iridomyrmecin-type. Thus, from *Alstonia boonei* De Wild (Apocynaceae), together with indole alkaloids, a δ-lactone of the nepetalactone-type, boonein (**1**) [5], was isolated. Its structure is closely related to that of loganin (**2**). In the present study of the iridoid content of *R. grandiflora* Mart, we have isolated loganin (**2**), loganic acid (**3**), and a mixture of boonein (**1**) and its corresponding iridomyrmecin-type isomer (**4**), i.e. isoboonein. The structure of isoboonein, assumed by spectroscopic analysis, has been confirmed by partial synthesis.



Boonein 1 Isoboonein 4

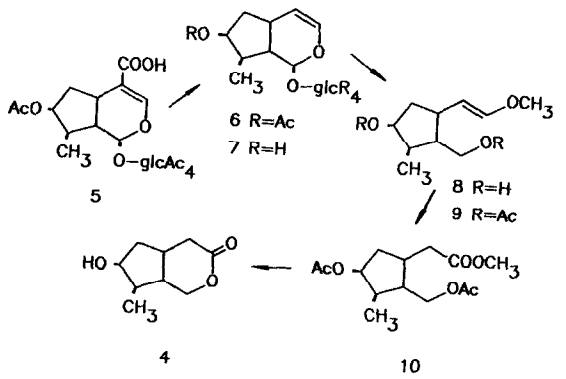
Loganin 2 R=CH_3
Loganic acid 3 R=H

RESULTS AND DISCUSSION

Isoboonein (**4**), $C_9H_{14}O_3$, is an iridolactone of the iridomyrmecin-type. In the ^{1}HNMR spectrum of **4** (see Experimental) there is an ABX system, attributable to the oxymethylene at C-1 (H-1a: δ4.32, *dd*, and H-1b: δ4.16, *dd*) and H-9 (δ2.16, *m*), as well as of an ABC system, assigned to H_2-4 (H-4a: δ2.66, *dd*, and H-4b: δ2.39, *dd*) and H-5 (δ2.95, *m*). The ^{1}H and ^{13}C NMR data (see Experimental) of the cyclopentane ring protons of **1** are very similar to the corresponding ones reported for loganin [6], boonein [5] and gibboside aglucone [7], and allow the assignment of the same substituents and relative configurations for this part of the molecule.

The six-membered ring of 5,9-*cis*-irido-3-lactones, due to the mobility of the lactone ring, can assume two antipodes of the boat conformation between the so-called iridomyrmecin-type and isoiridomyrmecin-type forms. In the ^{1}HNMR spectrum of **4**, $J_{1a,9}$ and $J_{1b,9}$ have similar values (4.0 and 3.0 Hz, respectively) and thus show that the iridomyrmecin-type form [8] is present, since higher and different values are expected for the other conformation. This situation is mainly due to the different position of the lactone function, which in the isoiridomyrmecin-type form is co-planar to the other parts of the molecule, whereas in the iridomyrmecin-type form it is folded and nearer to the cyclopentane ring. As an expected consequence, the chemical shift values of the carbonyl group in compounds with iridomyrmecin-type conformation are

*Part XV in the series 'Iridoids in Equatorial and Tropical Flora'. For Part XIV see ref. 1.



found at *ca* 172 ppm, whereas in the isoiridomyrmecin-type ones they have *ca* 176 ppm [9, 10].

The structure of (4a*S*,6*S*,7*S*,7a*S*)-6-hydroxy-7-methyl-1,4,4a,7a-tetrahydro-cyclopenta[c]pyran-3-one assigned to the non-glycosidic iridoid isoboonein (**4**) was confirmed by partial synthesis from loganic acid, **3**.

Pentaacetylloganic acid (**5**) was treated with copper powder in quinoline to obtain pentaacetyl-decarbomethoxyloganin (**6**). Hydrolysis of **6** with MeONa afforded decarbomethoxyloganin (**7**), which underwent reductive opening of the dihydropyrane ring by reaction with Tl^{2+}–$NaBH_4$ to give **8**. After acetylation to **9**, oxidation of the enol-ether function performed with PCC, afforded the diacetyl derivative **10**, which on alkaline hydrolysis underwent deacetylation and lactonization with subsequent formation of a compound identical in all respects to isoboonein (**4**).

In addition to their importance as biogenetic precursors, iridolactones have been studied for their interesting physiological activities, including insecticidal, bactericidal, feline-attracting and stimulatory effects [8]. Considering that *R. grandiflora* contains 0.08% of reserpine [11], this study confirms the co-occurrence of indole alkaloids with iridoid glycosides and non-glycosidic iridoids of related structures in Apocynaceae.

EXPERIMENTAL

^{1}HNMR data of glucose moieties are not reported; ^{1}H and ^{13}CNMR: 125 and 500 MHz, respectively; $[\alpha]_D$: 20°; MS: 70 eV, direct inlet.

Isolation of iridoid fraction. Rauwolfia grandiflora Mart. was collected in Brazil, Serra de Barriga, Alagoas State, and identified by Dr Jose Elias de Paula, Universidade de Brasilia. A voucher specimen (EP8123) is deposited at the Herbarium, Departamento de Botanica, Universidade de Brasilia. Powdered bark (1 kg) was extracted (×2) with EtOH (2 l) for 24 hr at room temp. and the extract, after preliminary sepn by the charcoal method [12], afforded 2 crude iridoid frs: the 1st one (2.7 g, fr. A), obtained by elution with 30% EtOH, and the 2nd one (7.7 g, fr. B) with 60% EtOH. Fr. A was chromatographed on cellulose powder in *n*-BuOH–MeOH–H_2O (7:3:1) giving loganic acid (**3**), (1.2 g) and loganin (**2**), (530 mg), all identified by comparison with authentic samples. Fr. B was chromatographed on silica gel in *n*-BuOH satd H_2O and afforded loganin (**2**), (1.0 g), together with a mixt. (3:1), (100 mg) of boonein (**1**), identified by comparison of spectral data, and isoboonein (**4**). For analytical purposes, isoboonein (**4**) was sepd from boonein (**1**) by HPLC on μBondapack C-18 in MeOH–H_2O (1:1). Boonein (**1**), ^{1}HNMR ($CDCl_3$): δ4.28 (1H, *ddd*, $J_{3a,3b}$ = 11.0 Hz, $J_{3a,4a}$ = 6.0 Hz, $J_{3a,4b}$ = 3.0 Hz, H-3a), 4.17 (1H, *ddd*, $J_{3b,4a}$ = 8.5 Hz, $J_{3b,4b}$ = 2.5 Hz, H-3b), 1.44 (1H, *m*, H-4a), 1.18 (1H, *m*, H-4b), 2.85 (1H, *m*, H-5), 2.04 (1H, *ddd*, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 8.0 Hz, $J_{6a,7}$ = 0.5 Hz, H-6a), 1.45 (1H, *ddd*, $J_{5,6b}$ = 11.0 Hz, H-6b), 4.10 (1H, *m*, H-7), 2.20 (1H, *ddq*, $J_{7,8}$ = 4.0 Hz, $J_{8,9}$ = 10.0 Hz, $J_{8,10}$ = 7.7 Hz, H-8), 2.63 (1 H, *t*, $J_{5,9}$ = 10.0 Hz, H-9), 1.19 (1H, *d*, $J_{8,10}$ = 8.0 Hz, H-10). Isoboonein **4** is a clear oil. $[\alpha]_D$ = +65.0° (MeOH; *c* 0.2). FAB-MS, *m/z*: 171 $[M+1]^+$: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1740, 1210; ^{1}HNMR ($CDCl_3$): δ4.32 (1 H, *dd*, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,9}$ = 4.0 Hz, H-1a), 4.16 (1 H, *dd*, $J_{1b,9}$ = 3.0 Hz, H-1b), 2.66 (1 H, *dd*, $J_{4a,4b}$ = 15.5 Hz, $J_{4a,5}$ = 7.5 Hz, H-4a), 2.39 (1H, *dd*, $J_{4b,5}$ = 4.0 Hz, H-4b), 2.95 (1H, *m*, H-5), 2.06 (1H, *ddd*, $J_{6a,6b}$ = 14.0 Hz, $J_{5,6a}$ = 8.0 Hz, $J_{6a,7}$ = 1.0 Hz, H-6a), 1.43 (1H, *ddd*, $J_{5,6b}$ = 11.0 Hz, $J_{6b,7}$ = 3.5 Hz, H-6b), 4.15 (1 H, *m*, H-7), 1.94 (1 H, *ddq*, $J_{7,8}$ = 4.0 Hz, $J_{8,9}$ = 10.0 Hz, $J_{8,10}$ = 8.0 Hz, H-8), 2.16 (1H, *m*, H-9), 1.09 (1H, *d*, $J_{8,10}$ = 8.0 Hz, H-10); ^{13}CNMR ($CDCl_3$): δ67.5 (C-1), 172.3 (C-3), 31.6 (C-4), 33.5 (C-5), 40.6 (C-6), 74.4 (C-7), 40.7 (C-8), 40.4 (C-9), 13.3 (C-10).

Decarboxylation of loganic acid. Loganic acid (**3**) (260 mg) was acetylated with Ac_2O (1 ml)–pyridine (0.5 ml) at room temp. overnight. The reaction mixt. was diluted with MeOH (5 ml), the volatile materials eliminated *in vacuo* and the residue chromatographed on μBondapack C-8 in MeCN–H_2O (1:1) to obtain pentaacetylloganic acid (**5**) (275 mg). Compound **5** is an oil. ^{1}HNMR ($CDCl_3$): δ5.19 (1H, *d*, $J_{1,9}$ = 2.0 Hz, H-1a), 7.31 (1H, *s*, H-3), 2.91 (1H, *br q*, $J_{5,9}$ = 8.5 Hz, $J_{5,6}$ = 8.5 Hz, H-5), 2.20 (2H, *m*, H-6), 5.17 (1H, *m*, H-7), 0.95 (3H, *d*, $J_{8,10}$ = 8.0 Hz, H-10); ^{13}CNMR ($CDCl_3$): δ95.0 (C-1), 151.0 (C-3), 113.0 (C-4), 29.4 (C-5), 38.5 (C-6), 77.2 (C-7), 38.6 (C-8), 45.1 (C-9), 12.1 (C-10), 171.9 (C-11), 96.0 (C-1′), 68.2 (C-2′), 72.0 (C-3′), 70.6 (C-4′), 72.4 (C-5′), 61.5 (C-6′), 170.8–169.3 and 20.6–20.0 (COMe). Compound **5** (100 mg) was dissolved in quinoline (2 ml), to which copper powder (5 mg) was added and the reaction refluxed for 3 hr. Thereafter, more copper powder (5 mg) was added and the refluxing continued for an additional 2 hr. The reaction mixt. was diluted with EtOAc (50 ml), the copper powder removed by filtration and the quinoline by washing with cold 2 M HCl. After the usual work-up, volatile materials were eliminated *in vacuo* and the residue chromatographed on silica gel in $CHCl_3$–MeO-*t*-Bu affording pentaacetyl decarbomethoxyloganin (**6**), (45 mg). Compound **6** is an oil. ^{1}HNMR ($CDCl_3$): δ5.19 (1H, *d*, $J_{1,9}$ = 4.0 Hz, H-1), 6.00 (1H, *dd*, $J_{3,4}$ = 6.2 Hz, $J_{3,5}$ = 2.1 Hz, H-3), 4.64 (1H, *dd*, $J_{4,5}$ = 3.0 Hz, H-4), 2.65 (1H, *m*, H-5), 1.90 (1H, *ddd*, $J_{6a,6b}$ = 15.0 Hz, $J_{5,6a}$ = 8.0 Hz, $J_{6a,7}$ = 3.0 Hz, H-6a), 1.80 (1H, *ddd*, $J_{5,6b}$ = 3.5 Hz, $J_{6b,7}$ = 6.0 Hz, H-6b), 5.13 (1H, *m*, H-7), 2.1–1.9 (1H, *m*, H-8 and H-9), 0.95 (3H, *d*, $J_{8,10}$ = 8.0 Hz, H-10); ^{13}C NMR ($CDCl_3$): δ93.4 (C-1), 137.3 (C-3), 108.6 (C-4), 28.9 (C-5), 38.2 (C-6), 77.1 (C-7), 37.4 (C-8), 45.6 (C-9), 12.1 (C-10).

Ring opening of **6**. Compound **6** (100 mg) was dissolved in MeOH (10 ml) and a 1 M methanolic soln of MeONa (1 ml) was added. After complete hydrolysis, the soln was neutralized by bubbling with CO_2. The volatile materials were eliminated *in vacuo* and the residue chromatographed on silica gel in $CHCl_3$–MeOH (9:1) to give decarbomethoxyloganin (**7**) (75 mg), as an oil. ^{1}HNMR (CD_3OD): δ5.17 (1H, *d*, $J_{1,9}$=3.5 Hz, H-1), 6.10 (1H, *dd*, $J_{3,4}$=6.2 Hz, $J_{3,5}$=2.1 Hz, H-3), 4.76 (1H, *dd*, $J_{4,5}$=3.0 Hz, H-4), 2.80 (1H, *m*, H-5), 1.70 (1H, *ddd*, $J_{6a,6b}$=15.0 Hz, $J_{5,6a}$=8.0 Hz, $J_{6a,7}$=3.0 Hz, H-6a), 1.62 (1H, *ddd*, $J_{5,6b}$=3.5 Hz, $J_{6b,7}$=6.0 Hz, H-6b), 4.09 (1H, *m*, H-7), 1.90 (1H, *m*, H-8), 1.97 (1H, *m*, H-9), 1.05 (3H, *d*, $J_{8,10}$=8.0 Hz, H-10);^{13}CNMR (CD_3OD): δ96.6 (C-1), 139.6 (C-3), 109.7 (C-4), 31.6 (C-5), 42.5 (C-6), 75.4 (C-7), 41.4 (C-8), 47.4 (C-9), 13.3 (C-10), 99.9 (C-1'), 71.8 (C-2'), 78.4 (C-3'), 75.0 (C-4'), 78.2 (C-5'), 62.9 (C-6'). Compound **7** (25 mg) was dissolved in MeOH (15 ml) and treated sequentially with stirring with $Tl(NO_3)_3$ (250 mg) and $NaBH_4$ (0.5 g). The soln was diluted with H_2O and neutralized by bubbling with CO_2. The MeOH was eliminated *in vacuo* and the aq. soln was treated with charcoal powder (300 mg). The charcoal was stratified on a gooch funnel, washed with H_2O until negative to salts and then with MeOH. After evapn of volatile materials *in vacuo*, the organic soln afforded a residue which was chromatographed on silica gel in $CHCl_3$–MeOH (24:1) affording pure **8** (12 mg) as an oil. $[\alpha]_D$=+58 (MeOH; *c* 0.2). ^{1}HNMR (CD_3OD): δ3.60 (2H, AB system, H-1), 6.31 (1H, *d*, $J_{3,4}$=12.5 Hz, H-3), 4.76 (1H, *dd*, $J_{4,5}$=10.0 Hz, H-4), 2.93 (1H, *m*, H-5), 1.6–2.0 (2H, *m*, $J_{6a,7}$=6.0 Hz, $J_{6b,7}$=1.2 Hz, H-6), 4.10 (1H, *dt*, H-7), 1.6–2.0 (2H, *m*, H-8 and H-9), 1.01 (3H, *d*, H-10), 3.50 (3H, *s*, OMe); ^{13}CNMR (CD_3OD): δ 63.5 (C-1), 148.3 (C-3), 104.4 (C-4), 37.2 (C-5), 42.7 (C-6), 75.8 (C-7), 40.9 (C-8), 49.2 (C-9), 13.4 (C-10), 56.6 (OMe).

Oxidation of **8**. Compound **8** (12 mg) was acetylated with pyridine (0.1 ml) and Ac_2O (0.2 ml) for 2 hr at room temp. After the usual work-up, chromatography on silica gel in benzene–Et_2O (19:1) afforded pure **9** (15 mg) as an oil. ^{1}HNMR ($CDCl_3$): δ4.08 (1H, *dd*, $J_{1a,1b}$=11.5 Hz, $J_{1a,9}$=7.5 Hz, H-1a), 3.98 (1H, *dd*, $J_{1b,9}$=5.0 Hz, H-1b), 6.26 (1H, *d*, $J_{3,4}$=12.5 Hz, H-3), 4.55 (1H, *d*, $J_{4,5}$=10.0 Hz, H-4), 2.83 (1H, *m*, H-5), 1.70 (1H, *ddd*, $J_{6a,6b}$=15.0 Hz, $J_{5,6a}$=8.0 Hz, $J_{6a,7}$=5.0 Hz, H-6a), 1.87 (1H, *ddd*, $J_{6b,7}$=2.5 Hz, $J_{6b,5}$=8.0 Hz, H-6b), 5.20 (1H, *m*, H-7), 1.6–2.0 (2H, *m*, H-8 and H-9), 0.96 (3H, *d*, $J_{8,10}$=8.0 Hz), 3.46 (3H, *s*, OMe). Compound **9** (15 mg) was dissolved in CH_2Cl_2 (4 ml) and treated, with stirring, with PCC (100 mg) for 6 hr. Excess PCC was destroyed with $Na_2S_2O_3$, the reaction mixt. diluted with Et_2O (50 ml) and the organic phase washed with H_2O until neutral. After evapn of volatile materials, the residue was chromatographed on silica gel in benzene–Et_2O (17:3) affording pure **10** (10 mg) as an oil. $[\alpha]_D$=+30 (MeOH; *c* 0.2) ^{1}HNMR ($CDCl_3$): δ4.07 (1H, *dd*, $J_{1a,1b}$=11.5 Hz, $J_{1a,9}$=3.8 Hz, H-1a), 3.94 (1H, *dd*, $J_{1b,9}$=7.0 Hz, H-1b), 2.24 (1H, *dd*, $J_{4a,4b}$=15.7 Hz, $J_{4a,5}$=6.5 Hz, H-4a), 2.51 (1H, *dd*, $J_{4b,5}$=11.0 Hz, H-4b), 2.78 (1H, *m*, H-5), 1.62 (1H, *ddd*, $J_{6a,6b}$=15.5 Hz, $J_{5,6a}$=8.0 Hz, $J_{6a,7}$=5.0 Hz, H-6a), 1.93 (1H, *ddd*, $J_{7,8}$=5.0 Hz, $J_{6b,7}$=1.5 Hz, H-6b), 5.18 (1H, *ddd*, H-7), 1.96 (1H, *m*, $J_{7,8}$=5.0 Hz, H-8), 2.13 (1H, *m*, $J_{5,9}$=8.0 Hz, H-9), 0.96 (3H, *d*, $J_{8,10}$=8.0 Hz, H-10), 3.65 (3H, *s*, OMe).

Alkaline hydrolysis of **10**. Compound **10** (10 mg) was dissolved in MeOH (0.5 ml), satd $Ba(OH)_2$ soln (0.5 ml) added and the soln allowed to stand at room temp. for 24 hr. CO_2 was bubbled into the soln which was diluted with MeOH (10 ml), until complete carbonate precipitation (pH 5). The salts were removed by filtration and the soln acidified (pH 1) with 2 M HCl. MeOH was eliminated by evapn *in vacuo* and the soln extracted with EtOAc. After removing volatile materials, the residue was chromatographed on silica gel in $CHCl_3$–MeOH (97:3) affording **4** (5 mg) as a solid.

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