IDENTIFICATION OF IRIDOID AND SESQUITERPENES FROM *Buddleia parviflora* BY NMR SPECTRA

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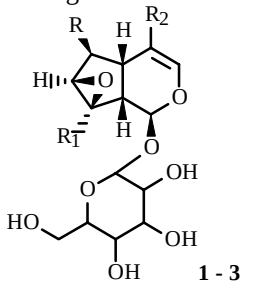
A new compound was elucidated as 9-acetyl-6-caryophyllen-15-ol. Iridoid glucosides including catalpol, methyl catalpol, 7-deoxy-8-epiloganic acid, and aucubin, were isolated from leaves of Buddleia parviflora, *while the known compounds were identified as dehydrobuddledin A and buddledin C. The structures was elucidated by extensive 1D-2D-NMR spectroscopy.*

Key words: *Buddleia parviflora*, iridoid glucoside, sesquiterpenes, NMR.

Buddleia parviflora H.B.K. belongs to the family Loganiaceae, subfamily buddleioideae. It is commonly know as «Santa Maria» and is used in traditional medicine for various types of illnesses. It is a common herb that grows wild and abundantly in the fields of Mexico. A water extract of the leaves has long been used by Guerrero natives for the treatment of wounds, and the use in Aguascalientes of powdered leaves to treat ulcers was reported a long time ago [1]. A decoction of the roots is drunk for the treatment of hepatitis. The leaves are also used as wound dressings to prevent suppuration [2]. *B. parviflora* is a plant that originated in Mexico and that has not been previously investigated for its chemistry and pharmacological effectiveness. Here we report the isolation of some iridoid glucosides and sesquiterpenes from *B. parviflora*.

The spectral data (IR, ^{1}H NMR and ^{13}C NMR) for methyl catalpol (**1**) [3–5], catalpol (2) [6], 7-deoxy-8-epiloganic acid (**3**) [7–10], aucubin (**4**) [2, 11], dehydrobuddledin A (**5**) [12], and buddledin C or 3(15),6-caryophylladien-8-one (**6**) [13] are in agreement with literature values.



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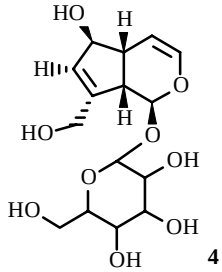

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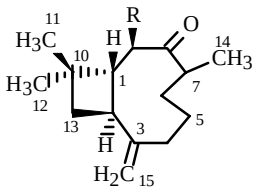

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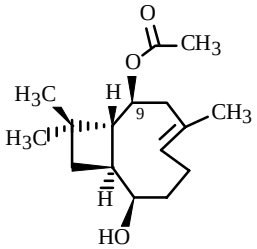

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1: R = β-OCH$_3$, R$_1$ = -CH$_2$OH, R$_2$ = H; **2:** R = OH, R$_1$ = -CH$_2$OH, R$_2$ = H; **3:** R = H, R$_1$ = CH$_3$, R$_2$ = COOH; **5:** R = CH$_3$COO; **6:** R = H, $\Delta^{6,7}$

The elucidation of the structure of the new compound was accomplished by extensive analyses of its spectral data. HPLC showed one main peak accompanied by two minor peaks. The corresponding substances were isolated by preparative HPLC. The compound corresponding to the main peak was denoted compound **7**. IR spectrum showed absorptions for acetoxyl (1743 and 1248 cm^{-1}), hydroxyl (3625 cm^{-1}), and conjugated carbonyl (1686, 1640) functionalities. The ^{1}H NMR spectrum in $CDCl_3$ exhibited signals for three singlet methyl groups (1.12, 1.11, and 1.66), one acetoxyl group (δ 2.23), one methine proton attached to an acetoxyl group (δ 5.22), a multiplet at 6.41 for the proton of the double bond, a methylene flanked by a double bond, and an ester group at δ 2.79. The chemical shift δ_H 3.65 was consistent with the hydroxy group being affixed to C-3 as observed in β-caryophyllene alcohol [14].

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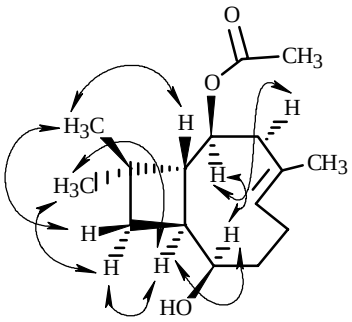

Fig. 1.

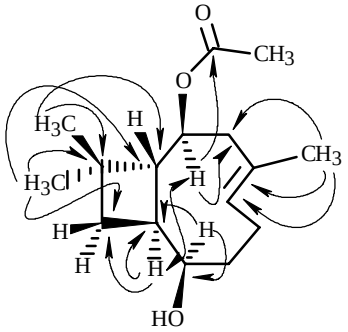

Fig. 2.

Fig 1. Correlations observed in the NOESY spectrum of compound **7**. The arrows indicate correlations between hydrogen and hydrogen (^{1}H–^{1}H).

Fig. 2. Correlations observed in the HBM spectrum of compound **7**. The arrows indicate correlations between carbon and hydrogen.

The ^{13}C NMR spectrum exhibited one acetoxy (δ 34.2, 174.1), one carbon attached to an acetoxyl group (δ 80.6), a double bond at δ 124.3 (C-7) and 139.6 (C-8). The carbon signal observed at δ 72.5 indicated the presence of a secondary hydroxyl group. The NOESY spectrum showing the connectivities of H-3 with H-2, H-13, and H-9 showed that the hydroxyl group at C-3 is *β*-oriented (equatorial). The ^{13}C NMR spectrum displayed signals for only 16 carbons, which were distinguished as four methyls (one vinylic, two aliphatic, and one acetyl), four methylenes (aliphatic), five methines (one vinylic, one hydroxyl, two aliphatic, one acetyl), and three quaternary carbons (one vinylic, one aliphatic, one carbonyl) with the aid of the DEPT experiments. The data suggested that compound **7** might be of a caryophyllene type [15, 16]. These fragments account for a partial molecular formula $C_{15}H_{26}O_3$, indicating that the three oxygens are present in hydroxyl and acetoxyl groups. The relative stereochemistry was established by NOESY experiments. NOESY correlations are shown in Fig. 1, and HMBC in Fig. 2. On the basis of the above evidence the structure of compound **7** was assigned as 9-acetyl-6-caryophyllen-15-ol.

EXPERIMENTAL

Plant Material. The leaves were collected in the state of Hidalgo, Mexico, and were taxonomically authenticated in the Department of Botany of ENEP-Iztacala UNAM. A voucher specimen (4879) of the plant is stored in the herbarium of this department for reference.

General Experimental Procedure. IR spectra were run in KBr on a Perkin Elmer 1710 spectrophotometer. All NMR experiments were performed on a Varian 300 Hz spectrometer. The HPLC apparatus was a Varian Mod. 9050.

Extraction and Isolation of Compound. The powdered, dried, aerial parts of *B. parviflora* (5 kg) were defatted with hexane and extracted successively with $CHCl_3$ and methanol. The chloroform and MeOH extract on concentration gave a brown viscous solid (280 and 198 g respectively). The methanol extract was chromatographed over silica gel using EtOAc – hexane (1:1) as eluent, and 9 fractions were collected. Fractions F6A and F7A were combined and separated by column chromatography on silica gel and eluted with Et OAc – $CHCl_3$ (7:1) to yield five secondary fractions. Further column chromatography on silica gel of fractions F-5B and F6B and eluting with $CHCl_3$ – MeOH - hexane (10 : 1.0 : 2) yielded six fractions each. Fractions F-2C and F-5C were column chromatographed over Sephadex LH-20 and eluted with $CHCl_3$ to yield four fractions. Finally, fractions F-3D and F-5D were further crystallized from methanol to yield 300 mg (**1**) and 150 mg (**2**). F-5C yielded 71 mg (**3**) and 69 mg (**4**) after separation on TLC [silica gel, $CHCl_3$ – MeOH, 9:3]. The chloroform extract was fractionated by chromatography over silica gel using hexane – ethylacetate (2 : 6) to yield six secondary fractions. Fractions 4C and 5C, which were composed of a sesquiterpenes mixture, were further purified by column chromatography and eluting with $CHCl_3$ – acetone (9:1), resultinging five fractions. Finally fractions 3D and 4D were column chromatographed over Sephadex LH-20 and eluted with chloroform to yield compounds **5** (30 mg), **6** (50 mg), and **7** (42 mg). Compound **7** was redissolved in 2 mL of methanol and

filtered through a 0.45 μm filter, and 10 μL of the filtrate was injected into an HPLC system. The column was a C-18 reverse phase (5 μm, 250 × 4.6 mm i.d; Merck), using 100% methanol as the mobil phase. The flow rate was 1 mL/min, and the peaks were detected with a L-4200 UV-vis detector set a 273 nm.

Acid Hydrolysis. Compound (0.1 g) was dissolved in 1N H_2SO_4, (5 mL) and refluxed for 6 h. The black degradation products were removed by filtration and the solution neutralized with $Ba(OH)_2$ (sat. sol.); the suspension was filtered, the solution evaporated, and the residue (40 mg) chromatographed on Si gel in $CHCl_3$ – MeOH (7 : 3) to give 35 mg of D-glucose identified by comparison with an authentic sample (R_f, ^{1}H NMR).

Methyl Catalpol (1). White prisms, mp. 190–192°C; Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 376.4091 $[M + H]^+$ (calcd for $C_{16}H_{24}O_{10}$ 376.4060); UV spectrum (MeOH, λ_{max}, nm): 214 (log ε 4.12); IR spectrum (KBr, ν, cm^{-1}): 3367 (OH), 3219, 1650 (enolic C=C), 1624 (C=C), 1410, 1386, 1363; ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6, J/Hz): 5.12 (d, $J_{1,9}$ = 2.5, H-1), 6.35 (dd, J = 2.5, 6.1, H-3), 5.02 (dd, J = 2.5, 6.1, H-4), 2.98 (dd, J = 2.5, 9.0, H-5), 4.52 (m, H-6), 3.28 (d, $J_{6,7}$ = 7.4, H-7), 3.81 (dd, J = 2.5, 9.0, H-9), 3.77 (bs, H-10), 3.75 (s, MeO), glucosyl: 4.25 (d, $J_{1',2'}$ = 7.5, H-1′), 3.14 (dd, $J_{2',3'}$ = 9.1, $J_{1',2'}$ = 7.9, H-2′), 3.37 (dd, J = 9.0, 8.7, H-3′), 3.26 (dd, J = 9.6, 8.6, H-4′), 3.31 (m, H-5′), 3.66 (dd, J = 11.9, 6.1, H-6′α), 3.90 (dd, J = 11.9, 2.1, H-6′β); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 93.73 (C-1), 141.37 (C-3), 103.58 (C-4), 46.78 (C-5), 46.28 (C-6), 61.97 (C-7), 59.41 (C-8), 42.41 (C-9), 61.97 (C-10), 52.1 (OMe), glucosyl: 98.44 (C-1′), 70.84 (C-2′), 78.13 (C-3′), 74.05 (C-4′), 77.02 (C-5′), 65.74 (C-6′).

Catalpol (2). White amorphous powder, mp 209–210°C; Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 362.4491 $[M + H]^+$ (calcd for $C_{15}H_{22}O_{10}$ 362.4345); UV spectrum (MeOH, λ_{max}, nm): 200 (log ε 4.52); IR spectrum (KBr, ν, cm^{-1}): 3358 (OH), 3220, 1655 (enolic C=C), 1623 (C=C); ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6, J/Hz): 5.42 (d, $J_{1,9}$ = 2.5, H-1), 6.45 (dd, J = 2.5, 6.2, H-3), 5.32 (dd, J = 2.5, 6.2, H-4), 2.96 (dd, J = 2.5, 9.2, H-5), 4.57 (dd, J = 1.4, 7.0, H-6), 3.28 (d, $J_{6,7}$ = 7.8, H-7), 3.81 (dd, J = 2.5, 9.2, H-9), 3.57 (bs, H-10), 3.95 (s, MeO), glucosyl: 4.27 (d, $J_{1',2'}$ = 7.5, H-1′), 3.16 (dd, $J_{2',3'}$ = 9.1, $J_{1',2'}$ = 7.9, H-2′), 3.39 (dd, J = 9.0, 8.7, H-3′), 3.23 (dd, J = 9.6, 8.6, H-4′), 3.30 (m, H-5′), 3.66 (dd, J = 11.9, 6.1, H-6′α), 3.91 (dd, J = 11.9, 2.1, H-6′β); ^{13}C NMR (100 MHz,δ, ppm, DMSO-d_6): 93.84 (C-1), 141.57 (C-3), 103.61 (C-4), 47.10 (C-5), 61.38 (C-6), 61.88 (C-7), 59.11 (C-8), 43.21 (C-9), 61.97 (C-10), 52.1 (OMe), glucosyl: 98.32 (C-1′), 70.14 (C-2′), 78.23 (C-3′), 74.14 (C-4′), 77.22 (C-5′), 65.75 (C-6′).

7-Deoxy-8-epiloganic Acid (3). White amorphous powder; mp 118–120°C; UV spectrum (MeOH, λ_{max}, nm): 209, 228; IR spectrum (KBr, ν, cm^{-1}): 3455 (OH), 1687 (enolic C=C), 1633 (C=C); Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 362.3854 $[M + H]^+$ (calcd for $C_{16}H_{22}O_{10}$ 374.3734); ^{1}H NMR (300 MHz, δ, ppm, DMSO-d_6, J/Hz): 5.15 (J = 9.6, H-1), 7.22 (br s, H-3), 2.81 (m, H-5), 1.41 (dd, J = 10.3, 13.8, H-6α), 2.63 (dd, J = 7.5, 13.8, H-6β), 3.29 (m, H-7), 2.18 (dd, J = 7.4, 9.6, H-9), 1.63 (s, H-10), glucosyl: 4.81 (1H, d, J = 7.9, H-1), 3.19-3.44 (4H, m, H-2′-5′), 3.66 (1H, dd, J = 5.8, 11.9, H-6′α), 3.93 (1H, dd, J = 1.9, 11.9, H-6′β); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 95.9 (C-1), 148.2 (C-3), 115.7 (C-4), 32.8 (C-5), 37.1 (C-6), 64.0 (C-7), 65.4 (C-8), 45.6 (C-9), 18.5 (CH_3-10), 174.1 (COOH), glucosyl: 99.9 (C-1′), 75.6 (C-2′), 78.3 (C-3′), 72.1 (C-4′), 78.7 (C-5′), 63.2 (C-6′).

Aucubin (4). White amorphous powder, mp 181–183°C; Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 346.341$[M + H]^+$ (calcd for $C_{15}H_{22}O_9$ 346.334); UV spectrum (H_2O, λ_{max}, nm): 199 (log ε 3.84); IR spectrum (KBr, ν, cm^{-1}): 3340, 1650; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.28 (d, J = 5.0, H-1), 6.37 (dd, J = 6.0, 1.5, H- 3), 5.25 (dd. J = 6.3, 3.7, H-4), 2.85 (br signal, H-5), 4.59 (br signal, H-6) 5.78 (br singlet, H-7), 3.24 (pseudotriplet, H- 9), 4.57 (br signal); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 96.82 (C-1), 142.41 (C-3), 106.71 (C-4), 46.11 (C-5), 82.34 (C-6), 130.18 (C-7), 147.76 (C-8), 47.879 (C-9), 61.44 (C-10), glucosyl: 99.97 (C-1′), 73.90 (C-2′), 78.056 (C-3′), 70.94 (C-4′), 77.61 (C-5′), 63.20 (C-6′).

Dehydrobuddledin A (5). Oil; Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 278.389 $[M + H]^+$ (calcd for $C_{17}H_{26}O_3$ 278.391); IR spectrum (KBr, ν, cm^{-1}): 1650, 1757, 1247; ^{1}H NMR (300 MHz, $CHCl_3$, δ, ppm, J/Hz): 2.11 (dd, J = 11.2, 10.3, H-1), 3.06 (m, H-2), 2.23–1.62 (m, CH_2-4, CH_2-5, CH_2-6, CH_2-13), 2.82 (m, H-7), 5.29 (d, J = 11.2, H-9), 1.14 (s, Me-11), 1.13 (s, Me-12), 1.12 (s, J = 6.5, Me-14), 4.86 (s, H-15), 2.12 (s, COOMe); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 56.9 (C-1), 39.4 (C-2), 157.8 (C-3), 31.8 (C-4), 27.0 (C-5), 30.4 (C-6), 43.9 (C-7), 197.1 (C=O), 80.1 (C-9), 33.9 (C-10), 35.0 (C-11), 20.6 (C-12), 32.4 (C-13), 14.3 (C-14), 115.7 (C-15), 30.4 (COOMe), 170.4 (COOMe).

Buddledin C or 3(15),6-Caryophylladien-8-one (6). Mp 130–131°C, Mass spectrum (FAB$^+$, *m/z*, I_{rel}, %): 218.179 $[M + H]^+$ (calcd for $C_{15}H_{22}O$ 218.173); IR spectrum (KBr, ν, cm^{-1}): 1688, 1684, 1650; ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.19 (dd, J = 11.1, 10.5, H-1), 3.13 (m, H-2), 2.31-1.51 (m, CH_2-4, CH_2-5, CH_2-13), 6.32 (m, H-6), 2.79 (d, J = 10.5, H-9), 1.09 (s, Me-11), 1.10 (s, Me-12), 1.66 (s, Me-14), 4.82 (s, H-15); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 59.08 (C-1),

38.8 (C-2), 151.2 (C-3), 32.1 (C-4), 26.7 (C-5), 144.0 (C-6), 137.2 (C-7), 205.4 (C=O), 48.76 (C-9), 34.6 (C-10), 32.6 (C-11), 21.3 (C-12), 33.0 (C-13), 14.8 (C-14), 115.7 (C-15).

9-Acetyl-6-caryophyllen-15-ol (7). Oil; Mass spectrum (FAB^+, *m/z*, I_{rel}, %): 254.278 $[M + H]^+$ (calcd for $C_{15}H_{26}O_3$ 254.270); IR spectrum (KBr, ν, cm^{-1}): 3625 (OH), 1743 (C=O), and 1640 (C=C), 1248; ^1H NMR (300 MHz, δ, ppm, DMSO-d_6, J/Hz): 2.14 (dd, J = 11.3, 10.3, H-1), 3.44 (m, H-2), 3.65 (m, H-3), 2.31-1.54 (m, CH_2-4, CH_2-5, CH_2-13), 6.41 (m, H-6), 2.79 (d, J = 10.3, H-8), 5.22 (m, H-9), 1.12 (s, Me-11), 1.11 (s, Me-12), 1.66 (s, Me-14), 2.23 (s, COOMe); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 59.0 (C-1), 42.1 (C-2), 72.5 (C-3), 34.8 (C-4), 29.9 (C-5), 37.8 (C-6), 124.3 (C-7), 139.6 (C-8), 80.6 (C-9), 33.7 (C-10), 31.2 (C-11), 33.7 (C-12), 39.6 (C-13), 16.8 (C-14), 174.1 (COOMe), 34.2 (COOMe).

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