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A NEW TRITERPENE FROM THE RESIN OF *BURSERA SIMARUBA*

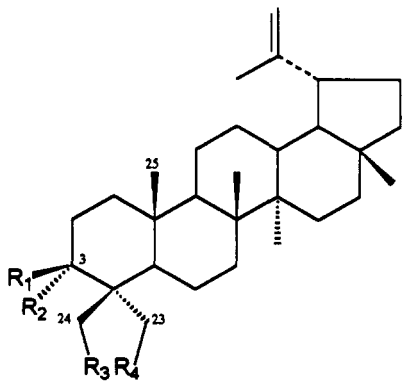
SERGIO R. PERAZA-SÁNCHEZ, NORMA E. SALAZAR-AGUILAR, and LUIS M. PEÑA-RODRÍGUEZ*

Departamento de Química Orgánica, Centro de Investigación Científica de Yucatán, A.C., Apartado Postal 87 Cordemex, Mérida, Yucatán, Mexico 97310

ABSTRACT.—A new lupane-type triterpene, lup-20(29)-en-3β,23-diol [**1**], has been isolated from the resin of *Bursera simaruba*, along with the known triterpenes α-amyrin, β-amyrin, lupeol, epilupeol, and epiglutinol. Structure determination of the new triterpene was based on an extensive analysis of its nmr and ms data.

We have described previously the isolation of the bioactive metabolite picropolygamain from the $CHCl_3$ extract of the resin of *Bursera simaruba* Sarg. (Burseraceae) (1), a tree commonly used in traditional medicine in the Yucatan to alleviate various diseases (2,3). Many species of the family Burseraceae owe their economic value to the essential oils, terpenes, steroids, and lignans in their resins (4). The resin triterpenes of plants in the Burseraceae can be broadly classified into four series, namely, the tetracyclic euphane/tirucallane type, and the pentacyclic lupane, ursane, and oleanane types (5). In our continuing studies on the components of the resin of *B. simaruba*, we wish to report herein the isolation and characterization of a new lupane derivative [**1**].

The known triterpenes lupeol, epilupeol, epiglutinol, α-amyrin, and β-amyrin were obtained in pure form during the isolation process. Compound **1** was purified as the corresponding benzoate, which, on alkaline hydrolysis, yielded **1** as a colorless wax exhibiting a molecular ion at *m/z* 442 (lreims) and a molecular formula of $C_{30}H_{50}O_2$. The DEPT nmr experiment permitted the assignment of all carbons in **1**, which comprised six methyl groups, twelve methylenes, six methines, and six quaternary carbons. In the ^{1}H-nmr spectrum of **1**, all methyl groups appeared as singlets, indicating their bonding to the six quaternary carbons. The low-field methyl signal at 1.65 ppm, together with the two protons of a terminal methylene group at 4.54 and 4.65 ppm, suggested the presence of an isopropenyl unit in **1** (6–8). Two sp^2 carbon signals at 109.3 (t) ppm and 150.9 (s) ppm in the ^{13}C-nmr spectrum of **1** confirmed the disubstituted alkene (8–12). The presence of an isopropylene unit, the number and type of methyl groups, and the significant fragments at *m/z* 229, 218, and 189 in the lreims of **1**, are all characteristic of compounds in the lup-20(29)-ene series (6–14). Evidence for the diol nature of both oxygen atoms of compound **1** came from its ^{13}C-nmr spectrum where two carbon signals, a methylene (72.15 ppm) and a methine (77.19 ppm), displayed chemical shifts characteristic for carbons bearing oxygen. The presence of one primary and one secondary alcohol in the structure of **1** was confirmed by the preparation of the corresponding diacetate [**2**]. Three signals observed in the ^{1}H-nmr



	R_1	R_2	R_3	R_4
1	OH	H	H	OH
2	OAc	H	H	OAc
3	H	OH	H	OH
4	OH	H	OH	H

spectrum of **1** at 3.59 (1H, dd, J=7.2 and 9.2 Hz), 3.39 (1H, d, J=10.4 Hz), and 3.69 ppm (1H, d, J=10.4 Hz), showed the expected downfield shifts, to 4.77, 3.69, and 3.85 ppm, respectively, in the ^{1}H-nmr spectrum of **2**. The magnitude of the acylation shifts observed is in agreement with those expected for protons on carbons bearing a secondary and a primary alcohol. On this basis, it was then possible to assign **1** with a lupene-type skeleton containing both a primary and secondary alcohol functionality.

The locations of the two hydroxyl groups in **1** were determined by analyzing the eims and ^{1}H- and ^{13}C-nmr data and comparing it to those of analogues reported in the literature. The methine proton appearing at 3.59 ppm (dd, J=7.2 and 9.2 Hz) was characteristic of triterpenes having an equatorial hydroxyl group at C-3 (8,15,16). The lreims of **1** displayed two prominent A/B ring fragments at *m/z* 223 [$C_{14}H_{23}O_2$] and 205 [*m/z* 223−H_2O], suggesting that the hydroxymethyl group could be located at either C-23, C-24, or C-25 (6,8). A survey of the literature showed that the ^{13}C-nmr signal for the primary alcohol carbon of **1** (72.15 ppm) is in agreement with that of a C-23-hydroxylated derivative (17–19), while the C-24-hydroxymethyl carbon consistently appears at higher fields (8, 19–21). There are reports in the literature indicating that a prominent $[M-31]^+$ fragment in triterpenes suggests the location of the hydroxymethyl group at an angular position (6,22). Since a $[M-31]^+$ fragment [*m/z* 411 (32%)] did appear in the lreims of **1**, it became necessary to confirm or rule out the presence of the primary hydroxyl group at C-25. An HMBC nmr spectral analysis of **1** showed a clear correlation between H-3 and the primary alcohol carbon, which could only be possible if the primary hydroxyl group was located at either C-23 or C-24. By comparing all the above data of **1** with those of the known lup-20(29)-ene-3α,23-diol [**3**] (6,18) and lup-20(29)-ene-3β,24-diol [**4**] (8), the structure of **1** was determined as lup-20(29)-en-3β,23-diol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded in $CHCl_3$ using a Perkin-Elmer 683 spectrophotometer. Mass spectra were recorded at 70 eV on a Kratos MS-50TC ultra-high resolution mass spectrometer. Gc-ms analyses were performed on a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5971A mass selective detector. ^{1}H- (200 and 400 MHz) and ^{13}C-nmr (50 and 100 MHz) spectra were determined on a Varian GEM-200 or a Unity-400 spectrometer, using $CDCl_3$ as the solvent and TMS as internal standard. Flash cc was run using Si gel 60 (200–400 mesh) from Aldrich Chemical Co; prep. tlc was carried out on Aldrich glass-coated Si gel 60 F-254 plates (0.25-mm thickness). Analytical tlc was conducted on precoated Si gel aluminum plates (Merck, Kieselgel 60 F-254, 0.2 mm thickness). Visualization was conducted under uv light and by dipping the plates in 4% phosphomolybdic acid containing a trace of ceric sulfate in 5% H_2SO_4, followed by drying and heating at 105° for 3–4 min.

PLANT MATERIAL.—As previously described by Peraza-Sánchez and Peña-Rodríguez (1).

EXTRACTION AND ISOLATION.—The resin (36.64 g) was collected and extracted as reported earlier (1). The crude resin extract (19.3 g, 52%) was subjected to successive flash cc purifications until purified fractions were obtained. Two fractions eluted from a hexane/Me_2CO purification were identified as epilupeol (39.6 mg) and epiglutinol (22.2 mg). Two other fractions, CHR-31A (55.0 mg) and CHR-31J (51.5 mg), showed the presence of two components with similar R_f values on tlc. In order to facilitate purification, both fractions were treated separately with 0.5 ml of benzoyl chloride and 1.0 ml of pyridine to afford the corresponding mixtures of benzoates. Purification by prep. tlc [hexane-$CHCl_3$ (9:1, 5×)] of the benzoylated CHR-31J (118.4 mg), followed by alkaline hydrolysis (1 ml of 20% KOH/MeOH) of the corresponding benzoate mixtures, yielded two fractions identified as lupeol (23.8 mg) and an inseparable mixture of α- and β-amyrin (10.0 mg). Lupeol and epilupeol were identified by direct comparison on tlc with authentic samples while the mixture of α- and β-amyrin was separated and identified by gc/ms. Epiglutinol was identified by comparing its ^{1}H-nmr and eims data with those reported for glutinol (23). The major component of fraction CHR-31A, identified as compound **1** (12.0 mg), was obtained in pure form

following prep. tlc purification [hexane-Et_2O(95:5, 5×)] and alkaline hydrolysis (1 ml of 20% KOH/MeOH) of the corresponding dibenzoate.

Lup-20(29)-en-3β,23-diol [**1**].—Colorless wax, R_f 0.29 [hexane-Me_2CO (9:1)]; ir ν max ($CHCl_3$) 3620 and 3450 (OH), 3020 (C-H stretch, olefinic), 1640 (C=C) cm^{-1}; lreims *m/z* $[M]^+$ 442 (94), 411 (32), 229 (21), 223 (68), 218 (58), 205 (60), 203 (50), 189 (50), 175 (31); ^1H nmr ($CDCl_3$, 200 MHz) δ 4.65 (1H, d, *J*=1.8 Hz, H-29), 4.54 (1H, d, *J*=1.8 Hz, H-29), 3.69 (1H, d, *J*=10.4 Hz, H-23), 3.59 (1H, dd, *J*=7.2 and 9.2 Hz, H-3), 3.39 (1H, d, *J*=10.4 Hz, H-23), 2.32 (1H, ddd, *J*=5.6, 10.6, and 11.2 Hz, H-19), 1.65 (3H, s, H-30), 1.00 (3H, s), 0.91 (3H, s), 0.85 (6H, s), 0.75 (3H, s); ^{13}C nmr ($CDCl_3$, 50 MHz) δ 35.55 (t, C-1), 27.07 (t, C-2), 77.19 (d, C-3), 42.84 (s, C-4), 50.90 (d, C-5), 18.45 (t, C-6), 34.03 (t, C-7), 40.80 (s, C-8), 50.41 (d, C-9), 37.99 (s, C-10), 20.91 (t, C-11), 25.09 (t, C-12), 37.05 (d, C-13), 42.84 (d, C-14), 27.42 (t, C-15), 35.55 (t, C-16), 42.98 (s, C-17), 47.97 (d, C-18), 48.26 (d, C-19), 150.93 (s, C-20), 29.83 (t, C-21), 39.98 (t, C-22), 72.15 (t, C-23), 11.21 (q, C-24), 15.98 (q, C-25), 16.45 (q, C-26), 14.56 (q, C-27), 18.02 (q, C-28), 109.33 (t, C-29), 19.30 (q, C-30).

ACETYLATION OF **1**.—Treatment of **1** (5.5 mg) with a mixture of Ac_2O and pyridine (1 ml each) resulted in the formation of 6.1 mg of the corresponding diacetate [**2**]. Colorless wax; ir ν max ($CHCl_3$) 3020 (C-H stretch, olefinic), 1735 and 1725 (C=O, acetate), 1645 (C=C) cm^{-1}; ^1H nmr ($CDCl_3$, 200 MHz) δ 4.77 (1H, dd, *J*=5.2 and 10.8 Hz, H-3), 4.69 (1H, d, *J*=2.2 Hz, H-29), 4.57 (1H, br d, *J*=0.9 Hz, H-29), 3.85 (1H, d, *J*=11.3 Hz, H-23), 3.69 (1H, d, *J*=11.7 Hz, H-23), 2.07 (3H, s, $MeCO_2$), 2.02 (3H, s, $MeCO_2$), 1.68 (3H, s, H-30), 1.03 (3H, s), 0.94 (3H, s), 0.89 (3H, s), 0.81 (3H, s), 0.78 (3H, s); ^{13}C nmr ($CDCl_3$, 50 MHz) δ 38.02 (t, C-1), 23.13 (t, C-2), 77.01 (d, C-3), 40.82 (s, C-4), 65.41 (d, C-5), 17.98 (t, C-6), 33.89 (t, C-7), 40.58 (s, C-8), 50.45 (d, C-9), 36.93 (s, C-10), 21.26 (t, C-11), 25.06 (t, C-12), 38.02 (d, C-13), 42.79 (d, C-14), 27.39 (t, C-15), 35.53 (t, C-16), 43.00 (s, C-17), 47.97 (d, C-18), 48.26 (d, C-19), 150.94 (s, C-20), 29.81 (t, C-21), 39.98 (t, C-22), 74.54 (t, C-23), 12.94 (q, C-24), 15.95 (q, C-25), 16.60 (q, C-26), 14.44 (q, C-27), 18.00 (q, C-28), 109.38 (t, C-29), 19.28 (q, C-30), 20.97 (q, *Me*CO_2), 20.97 (q, *Me*CO_2), 170.70 (s, Me*C*O_2), 171.09 (s, Me*C*O_2).

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