Two New Dammarane Triterpenoids from *Kageneckia angustifolia* D. Don

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Abstract: Two new dammarane triterpenoids, 20*R*,21-epoxydammar-24-ene-3,23-dione and 20R,21-epoxy-3β-hydroxydammar-24-ene-23-one have been isolated from the aerial parts of *Kageneckia angustifolia* D. Don., Rosaceae, along with the previously reported triterpenoids oleanolic acid and 3β-(β-*D*-glucosyloxy)-16α,23α-epoxycucurbita-5,24-dien-11-one and the phenolic prunasin. The structures of these compounds were established by MS, 1D- and 2D-NMR experiments, and the structure of the new compounds were confirmed by X-ray diffraction analysis.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; HMQC; HMBC; COSY; ROESY; *Kageneckia angustifolia*; Rosaceae; triterpenoids; dammaranes

INTRODUCTION

In our ongoing program to search for new natural bioactive compounds and their medicinal applications, a bioassay-guided study has been carried out with extracts from the aerial parts of *Kageneckia angustifolia* D. Don, a tree locally known as "olivillo" that grows in the central area of Chile.[1] Neither phytochemical nor pharmacological and toxicological studies have been previously reported about this plant. The selection of this plant has been based on the use of the related species *Kageneckia oblonga* R. et P. in traditional medicine, for the treatment of hepatic and kidney disorders and others ailments.[2,3]

Preliminary pharmacological evaluations carried out by us with *K. angustifolia* extracts showed analgesic activity. We also performed a comparative study of the analgesic effect and acute toxicity of the different extracts of both *Kageneckia* species.[4] Besides, the extracts from both species do not displayed cytotoxicity. Other pharmacological studies are presently undertaken and will be published soon.

Bioassay-guided fractionation of the crude hexane extract yielded the two new dammaranes triterpenoids **1** and **2**, whereas a similar fractionation led to the isolation of oleanolic acid from the dichloromethane extract and the cucurbitacin glycoside from the methanolic extract. In the present paper, the structure elucidation of the two new dammaranes compounds is reported.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white crystalline solid. HRFABMS of **1** showed $[M]^{+}$ at *m*/*z* 454.3454, which in combination with DEPT and BB ^{13}C NMR data allowed us to determine its

molecular formula as $C_{30}H_{46}O_3$. The IR spectrum of **1** displayed absorptions of instauration (1617 cm^{-1}) and two intense bands in the carbonyl region (1704 and 1686 cm^{-1}). The ^{13}C NMR spectrum displayed two signals at δ 197.3 and 218.0 ppm corresponding to two carbonyl groups. The former was conjugated with a double bond (156.2, 124.3 ppm), corroborated by the absorption at 1686 cm^{-1} in the IR spectrum. This information, together with the presence in the ^1H NMR spectrum of two singlets at δ 2.14 and 1.89 ppm, assigned to two vinyl methyls and a broad singlet at d 6.13 ppm of the α-proton of the α,β-unsaturated carbonylic moiety, indicated the presence of the $R_1COCH=CMe_2$ structural subunit[5], which forms part of the side-chain of this compound.

The presence of the dammarane skeleton in compound **1** was deduced from an iterative search carried out within the ^{13}C NMR data of a home-made database.[6] This database is under continuous building construction and currently contains ^{13}C NMR data of several families of natural compounds, mainly triterpenoids. This search also revealed the presence of a ketone group at position 3 in the structure of compound **1** by means of a comparison with the spectral data of several triterpenoids found during the search[7]. Nevertheless, the overlapping of a number of signals in the ^1H NMR spectrum did not allow performing the complete analysis of several absorptions important for the structural determination. Consequently, some 2D-NMR experiments (HMQC, COSY, HMBC and ROESY) were run in order to achieve more information about this compound. Table 2 summarizes the most important NMR data and the connectivities detected in the two-dimensional spectra.

The results from these experiments allowed the determination of the carbon connectivity of **1**, and herewith confirmed the presence of the dammarane skeleton. These experiments also allowed the assignment of all of the signals present in carbon and proton spectra except for three methylenes which were assigned by comparison with those of other similar triterpenoids described in the literature[8].

A good starting point for the analysis of the 2D-NMR experiments HMQC and HMBC was the olefinic methine at δ 124.3 ppm. This signal is connected with that of the vinyl methyls at δ 2.14 and 1.89 ppm, assigned to the terminal side-chain C-26 and C-27. In consequence, the signal at δ 124.3 was assigned to C-24. Additionally, this signal exhibited a long-range correlation with that of a methylene at 48.1 ppm, assigned to C-22. H-24 (6.13 ppm) was connected to the ketonic carbonyl signal at δ 197.3 ppm, which was assigned to C-23. Furthermore, an AB system centred at δ 2.51 and 2.81 (J=14.9 Hz) and assigned to H-22 was connected simultaneously, apart from those of C-23 and C-24, with a non-protonated carbon signal at δ 59.2 ppm assigned at C-20, with a methylene signal at δ 51.5 ppm and with a methine signal at δ 45.6 ppm that should be C-17. In consequence, the signal at δ 51.5 ppm must correspond to C-21, which is a methylene and not a methyl normally found in the dammaranes triterpenoids type. The methylene C-21 should be attached to the C-20 across an epoxy bridge. C-20, apart from H-22 and H-21, is connected with two methines that must to be H-17 and H-13. All these correlations demonstrate that the compound contains a dammarane skeleton, since other similar triterpenoid like lanostane, cucurbitane or tullicane derivatives have a methyl

group attached to C-13, which in the case of compound **1** is a non-protonated carbon. The rest of the long-range correlations observed appear in table 2 and are in complete agreement with this structure.

The configuration at C-17 was determinated from the rOe correlation observed between the alpha methyl protons H-18 and the alpha methine proton H-17. Furthermore, the beta disposition of H-13 was deduced from the rOe correlation connecting the signal of this proton with that of the methyl H-30, which in turn showed a rOe with H-19. Nevertheless, the configuration at C-20 could not be established by means of the rOe experiments described here and remained unclear. In order to establish the configuration at C-20 and to confirm the structure of **1**, a X-ray crystallographic diffraction analysis was carried out that allowed to solve both questions, thus confirming the structure of compound **1** as 20*R*,21-epoxydammar-24-ene-3,23-dione, a new natural compound.

Compound **2** was isolated as a white crystalline solid. HRFABMS of 2 showed $[M+H]^+$ at m/z 457.3673, which in combination with DEPT and BB ^{13}C NMR data allowed to determine its molecular formula as $C_{30}H_{48}O_3$. The IR spectrum exhibited absorptions indicative of a hydroxyl group (3382 cm^{-1}), a ketonic conjugated carbonyl (1684 cm^{-1}) and a double bond (1617 cm^{-1}). The ^1H and ^{13}C RMN spectra showed signals similar to those of compound **1,** particularly those corresponding to the side-chain moiety. Nevertheless, for compound **2** the signal at δ 218.0 ppm was absent. Instead, could be observed a signal at δ 78.9 ppm of an oxygenated methine. This was corroborated by the presence in the ^1H RMN spectrum of a double doublet centred at δ 3.18 ppm (J = 9.6; 5.9). This data were in agreement with the molecular formula and the IR spectrum, and indicated the presence of an equatorial hydroxyl group instead of the ketone group present in compound **1**. This proposal was confirmed by means of a similar iterative searching of ^{13}C RMN chemical shift data in the above-mentioned database.[6] The search allowed to locate the hydroxyl group at position 3 in beta disposition due to the similarity of ^{13}C chemical shift the rings A and B of compound **2** with those of the a considerable number of similar triterpenoids found during the search. In consequence, this compound should be 20,21-epoxy-3β-hydroxydammar-24-ene-23-one. The treatment of the hydroxylated compound **2** with pyridinium dichromate yielded a ketone identical to **1**. Besides, the structure of this new natural compound was reconfirmed by X-ray diffraction.

Although the side chain spectral data for both compounds are practically identical, a different spatial arrangement for compounds **1** (Figure 2) and **2** (Figure 3) can be observed for the X-ray diffraction models of their crystalline structures. In the solid state the A ring of the triterpenoid skeleton is in the common chair disposition in compound **2**, whereas in compound **1**, due to the presence of the 3-ketone group, is in a boat conformation. Furthermore, a completely different orientation can be observed in the side-chain in both compounds. In compound **1** this moiety is in an extended disposition, whereas in compound **2**, is over the polycyclic system of the triterpenoid. This disposition is forced by the establishment of an intermolecular hydrogen bond between the epoxide oxygen at position 20 of one molecule and the hydroxyl group at position 3 of the contiguous molecule within the crystalline lattice

(Figure 3). This situation cannot exist in the case of compound **1**.

EXPERIMENTAL

General procedures

Melting points were determined on a Büchi 510-K melting point apparatus and are uncorrected. IR spectra were recorded (KBr 1%) in a Nicolet Impact 410 spectrophotometer. Sephadex LH-20 (Fluka, 25–100 mm) and silica gel 60 (Merck, 230–400 mesh) were used for flash chromatography; precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis. ^{1}H, ^{13}C NMR, COSY, HMQC and HMBC were recorded in $CDCl_3$ solutions at 300 K on Bruker AC 200 (200 MHz) and Bruker DRX 400 (400 MHz) instruments. TMS was used as an internal reference for ^{1}H and ^{13}C. Resonance multiplicities for ^{13}C signals were established via the acquisition of DEPT spectra. For two-dimensional experiments, Bruker software using gradient selection (gs) were applied. gs-COSY spectra[9] were obtained with an F_2 spectral width of 10 ppm and 2 K data points and an F_1 spectral width of 256 t_1 increments with sine-bell windows in both dimensions. The gs-HMQC spectra[10] resulted from 256 x 1024 data matrix size with 2–16 scans per t_1 depending on the sample concentration, an inter-pulse delay of 3.2 ms and a 5:3:4 gradient combination. gs-HMBC spectra[11] were measured using a pulse sequence optimized for 10 Hz (interpulse delay for the evolution of long-range couplings 50 ms) and the same gradient ratios. For EIMS and HRFABMS analysis, a VG-TS250 mass spectrometer (70 eV) was used. X-ray diffraction data were collected on a four-circle Seifert XRD 3003 SC diffractometer (CuF_{α}, λ=1.5418 Å), graphite monochromator, room temperature, ώ-2θ scans. All calculations were performed using CRYSOM[12] software for data collection, XRAY80[13] for data reduction, SHELXTLTM[14] to resolve and refine the structure and to prepare figures for publication.

Plant Material

The aerial part of *K angustifolia* was collected in Cajón del Maipo, Santiago, Chile and identified by Dr. Carla Delporte. A voucher specimen is deposited in the Herbarium of the School of Chemistry and Pharmacy (SQF Nº 22144a), University of Chile.

Extraction and isolation

The air-dried and ground aerial part of *K angustifolia* (2.657 Kg) was successively extracted at room temperature with hexane, CH_2Cl_2 and MeOH yielding 154g, 160g and 790g of extract respectively after concentration *in vacuo*. Most parts of extracts and fractions were used in bioassays. Aliquots of the bioactive fractions were chromatographied. Thus from the hexane extract 61mg of compound **1** and 84 mg of compound **2** were obtained from two fractions eluted with hexane-CH_2Cl_2 7:3 and hexane-CH_2Cl_2 7:4 respectively. Both of them were further purified by successive crystallizations from $CHCl_3$–MeOH. From the CH_2Cl_2 extract, a small amount of oleanolic acid was isolated, purified and identified by comparison with an autentical sample with spectroscopy data identical to that reported in the literature.[15] From the global

methanol, the 3β-(β-D-glucosyloxy)-16α,23α-epoxycucurbita-5,24-dien-11-one and prunasin were identified and further corroborated by comparison with authentically samples previously isolated by us from *Kageneckia oblonga*.[16]

20R,21-epoxydammar-24-ene-3,23-dione (**1**): colourless crystals; mp 165-167 ºC; IR (KBr) γ_{max} 2947, 2871, 1704, 1686, 1746,1617, 1448, 1381, 1108, 924, 838 cm^{-1}; ^{1}H and ^{13}C NMR data: see Table 1; EIMS *m/z* 436 $[M - OH]^+$ (42), 421(5), 305(5), 281(5), 267(4), 229(5), 207(8), 201(10), 148(100), 123(15) 83(75); HRFABMS *m/z* 454.3454 (calcd. for $C_{30}H_{46}O_3$, 454.3447).

X-ray analysis of compound 1. Compound **1**, $C_{30}H_{46}O_3$. Crystal dimensions 0.10 x 0.25 x 0.30; crystallizes in orthorhombic space group $P2_12_12_1$, with Z=4, and unit cell parameters, a = 7.0240(14) Å, b = 13.385(3) Å, c = 27.656(6) Å, α = 90(1), β = 90(2), γ = 90(1)º. The unit cell parameters were determined by least squares refinement on the 2θ values of 25 strong well centred reflections in the range 3.20º <2θ <59.97º. Scattering factors for neutral atoms and anomalous dispersion correction for C and O were taken from "International Tables for X Ray Crystallography"[17]. The structure of $C_{30}H_{46}O_3$ was resolved by direct methods and refined in the space group $P2_12_12_1$. Full matrix least-squares refinement with anisotropic thermal parameters for non-H atoms was carried out by minimizing $w(Fo^2–Fc^2)^2$. Refinement on F^2 for all reflections, weighted R factors (R_w), and all goodness of fit S are based on F^2, while conventional R factors (R) are based on F; R factors based on F^2 are statistically about twice as large those based on F, and R factors based on all data will be even larger. Resulting absolute structure parameter: 0.18(45). Full crystallographic details have been deposited at the Cambridge Crystallographic Data Centre No. CCDC 2506931.

20R,21-epoxy-3β-hydroxydammar-24-ene-23-one (**2**): colourless crystals; mp 171-172 ºC; IR (KBr) γ_{max} 3448, 2948, 2872, 2360, 1684, 1617, 1446, 1377, 119, 1074, 1045, 985, 924 cm^{-1}; ^{1}H and ^{13}C NMR data: see Table 1; EIMS *m/z* 438 $[M - OH]^+$ (20), 436(5), 420(25), 405(5), 377(8), 341(4), 281(4), 229(4), 215(10), 201(12), 148(100), 83(75); HRFABMS *m/z* 456.3673 (calcd. for $C_{30}H_{48}O_3$, 456.3603).

X-ray analysis of compound 2. Compound **2**, $C_{30}H_{48}O_3$. Crystal dimensions 0.06 x 0.08 x 0.08; crystallizes in orthorhombic space group $P2_12_12_1$, with Z=4, and unit cell parameters, a=8.731(2) Å, b=10.113(2) Å, c=30.046(6) Å, α=90(1), β= 90(2), γ= 90(1)º. The unit cell parameters were determined by least squares refinement on the 2θ values of 25 strong well centred reflections in the range 1.47º <2θ < 59.90º. Scattering factors for neutral atoms and anomalous dispersion correction for C and O were taken from "International Tables for X Ray Crystallography"[17]. The structure of $C_{30}H_{48}O_3$ was resolved by direct methods and refined in the space group $P2_12_12_1$. Full matrix least-squares refinement with anisotropic thermal parameters for non-H atoms was carried out by minimizing $w(Fo^2–Fc^2)^2$. Refinement on F^2 for all reflections, weighted R factors (R_w), and all goodness of fit S are based on F^2, while conventional R factors (R) are based on F; R factors based on F^2 are statistically about twice as large those based on F, and R factors based on all data will be even larger. Resulting absolute structure

parameter: -5.18(297). Full crystallographic details have been deposited at the Cambridge Crystallographic Data Centre No. CCDC 2506931.

Oxidation of compound 2

To a stirred solution of **2** (15 mg) in CH_2Cl_2 (1 mL), pyridinium dichromate (25 mg) was added and left at room temperature for 3 h. Workup of the crude product by chromatography on silica gel led to the isolation of **1** (10 mg) identical to the isolated natural product.

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