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First examples of tetracyclic triterpenoids with a *D*:*B*-friedobaccharane skeleton. A tentative biosynthetic route

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This paper is dedicated in memoriam to Professor Antonio González González

Abstract—Baruol (**1**) and Leonal (**2**), first examples of tetracyclic triterpenes possessing a *D*:*B*-friedobaccharane skeleton, were isolated from *Maytenus blepharodes* and *M. chiapensis*, respectively. Their structures were established by spectroscopic analysis, molecular modeling studies and biogenetic background. The implication of the *D*:*B*-friedobaccharenyl cation in the biosynthetic route of baccharane and shionane skeletons is discussed. Baruol exhibited β-glucuronidase inhibitory activity, a target in the search for hepatoprotective agents.
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The species of the Celastraceae have a long history in traditional medicine, and they produce an extraordinary variety of bioactive metabolites. Triterpenoids from the Celastraceae belonged to the lupane, oleane, friedelane, taraxerane, glutinane, ursane, and dammarane series.[1] Reports on triterpenoids of the baccharane type are scant in the field of natural products and with few exception, restricted to terrestrial plants.[2] Hosenkol-A, with a pentacyclic skeleton, and sasanquol, a 3,4-*seco*-tricyclic triterpene, were the first occurring baccharane and *D*:*B*-friedobaccharane triterpenoids, reported.[3] Here we inform about the isolation and structure elucidation of two triterpenes (**1** and **2**) (Fig. 1) from *Maytenus blepharodes* and *M. chiapensis*, which we have named Baruol and Leonal, respectively.[4] To the best of our knowledge, they represent the first examples of tetracyclic triterpenes possessing a *D*:*B*-friedobaccharane skeleton. *D*:*B*-friedobaccharenyl cation (Scheme 1) had been postulated as an intermediate between baccharane and shionane series,[3] but no compound that could justified this had been isolated up to now. Baruol (**1**) exhibited β-glucuronidase inhibitory activity (57% of inhibition at 10 μg/mL), which is correlated to hepatoprotective activity.[5]

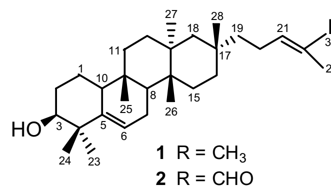

Figure 1. Structures of Baruol (**1**) and Leonal (**2**).

Baruol (**1**)[6] ($[\alpha]_D^{20}$ + 14, *c* 1.26, $CHCl_3$) was purified after repeated chromatography of the *n*-hexane/Et_2O (1:1) extract of the root bark of *M. blepharodes* on silica gel and Sephadex LH-20, and final purification was achieved by HPTLC using *n*-hexane/Et_2O (1:1) as eluent. Its HREIMS gave the molecular formula as $C_{30}H_{50}O$ (M^+, *m*/*z* 426.3818, calcd 426.3862), which implied six degrees of unsaturation accounted by two double bonds and four rings. The IR spectrum revealed the presence of a hydroxyl group (3438 cm^{-1}). Its NMR

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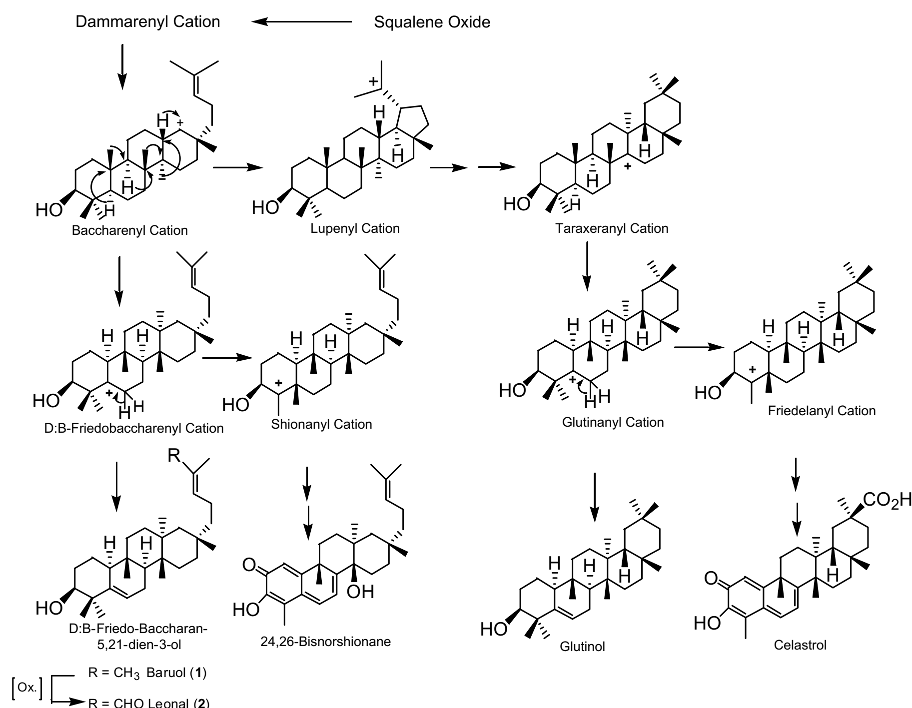

Scheme 1. Proposed biosynthetic route for the formation of Baruol (**1**) and Leonal (**2**).

data (Table 1) showed the presence of eight methyl groups, one secondary alcohol (δ_H 3.47, br t, and δ_C 76.3 ppm, H-3), two trisubstituted double bonds (δ_H 5.10, t, $J = 6.8$ Hz, δ_C 125.3 ppm, and δ_H 5.61, t, $J = 3.3$ Hz, δ_C 122.0 ppm), in addition to 10 methylenes, 2 methines, and 7 quarternary carbons. These data, along with analysis of the EIMS fragmentation (Fig. 2), and comparison with the NMR data of glutinane triterpenes[7] suggested that **1** was a tetracyclic triterpene with two trisubstituted double bonds and one secondary alcohol.

The full assignments and connectivities were determined by ^{1}H–^{1}H COSY, HSQC, and HMBC spectra. The hydroxyl group was placed at C-3 from the HMBC correlations, which linked the signal at δ_H 3.47 ppm to C-1 (δ_C 18.1) and C-5 (δ_C 142.0). The double bonds were sited at C-6 and C-21 as correlations linked the signal at δ_H 5.61 to C-4 (δ_C 40.8), C-7 (δ_C 23.7), C-8 (δ_C 44.7), and C-10 (δ_C 50.0), and the signal at δ_H 5.10 to C-29 (δ_C 17.6) and C-30 (δ_C 25.7) (Table 1). The relative configuration at C-3 and C-17 was solved by a ROESY experiment, showing NOE correlations to H-3$_{eq}$ to H-23 and H-24, and H-19 to H-27, and by the *J*-value for H-3 (br t, $\omega_{1/2} = 2.8$ Hz), which was supported by molecular modeling studies.[8] All these data, and biogenetic background[9] allowed us to propose the structure of Baruol (**1**) as *D*:*B*-friedobaccharan-5,21-dien-3-ol.

The molecular formula of Leonal (**2**)[10] (Fig. 1) was established as $C_{30}H_{48}O_2$ (M^+, *m/z* 440.3651, calcd 440.3654). Its spectroscopic data (IR, UV, ^{1}H, and ^{13}C data and 2D experiments) showed **2** to be related to **1**, with the most notable differences being the presence of an aldehyde group (δ_H 9.38 and δ_C 195.3 ppm), which

Table 1. NMR[a] spectral data (δ, $CDCl_3$) for Baruol (**1**)

No.	δ_H (mult, *J* in Hz)	δ_C[b]	HMBC
1	1.48	18.1	
2	1.71, 1.86	27.8	
3	3.47 br t (2.8)	76.3	1, 4,[c] 5
4		40.8	
5		142.0	
6	5.61 t (3.3)	122.0	4, 7,[c] 8, 10
7	1.85	23.7	
8	1.44	44.7	
9		35.6	
10	2.08	50.0	
11	1.34, 1.65	34.2	
12	1.58, 1.60	32.8	
13		36.5	
14		38.0	
15	1.22, 1.32	29.3	
16	1.53, 1.61	34.6	
17		31.9	
18	1.18, 1.69	44.5	
19	1.14, 1.24	43.3	
20	2.00	23.2	
21	5.10 t (6.8)	125.3	21, 22
22		130.8	29, 30
23	1.04	29.0	3, 4,[c] 5, 24
24	1.14	25.4	3, 4,[c] 5, 23
25	0.91	17.5	8, 9,[c] 10, 11
26	0.98	15.2	8, 13, 14,[c] 15
27	1.07	20.2	12, 13,[c] 14, 15
28	0.89	32.9	16, 17,[c] 18, 19
29	1.60	17.6	21, 22,[c] 30
30	1.68	25.7	21, 22,[c] 29

[a] Data collected at 400 MHz (^{1}H) and 100 MHz (^{13}C).
[b] ^{13}C multiplicities were assigned from DEPT or ^{1}H, ^{13}C-HSQC experiments.
[c] Two-bond coupling enhancement observed.

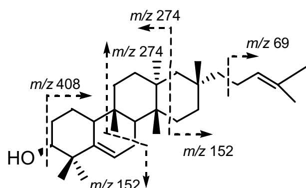

Figure 2. Intensive fragment peaks in EIMS of Baruol (**1**).

correlated to C-21 (δ_C 155.5), C-22 (δ_C 138.9), and C-29 (δ_C 9.1) in the HMBC experiment, and the absence of the methyl group on a double bond at δ_H 1.68 (H-30). The relative configuration at C-3 and C-17 was solved by a ROESY experiment, and that of the aldehyde group was determined by a GOESY experiment, showing NOE effect between H-21 (δ_H 6.48) and H-30 (δ_H 9.38). The above data led us to propose the structure of **2** as *D*:*B*-friedobaccharan-5,21-dien-3-ol-30-al.

The plant triterpenoids are biogenetically originated from oxidosqualene, through carbocationic intermediates.[9] The biogenesis of baccharane and shionane skeletons has been conceived as originated from dammarenyl cation via Friedo rearrangement (Scheme 1). Expansion of the D-ring of this cation is envisaged to furnish the baccharenyl cation, which by further cyclization, involving the olefinic bond, generated the lupenyl cation. An alternative pathway available implied the *D*:*B*-friedobaccharenyl cation; further methyl shift of this cation leads to shionane skeleton. On the other hand, deprotonation from C-6 gives *D*:*B*-friedobaccharan-5,21-diene triterpenes.

Celastraceae are able to biosynthesize, among others, pentacyclic (friedelane, glutinane) and tetracyclic (*D*:*B*-friedobaccharane) triterpenes with identical substitution partners in the B, C, and D rings. Up to now, the *nor*-triterpene methylene quinones isolated from Celastraceae (e.g., celastrol) have a friedelane skeleton. Russulaflavidin, a 24,26-bisnorshionane,[11] with the same chromophore group than the Celastraceae quinones, was recently isolated from *Russula flavisa* (Agaricales). This compound could be considered as being formally derived from shionanyl cation, which is in the biosynthetic route of the baccharenyl cation, this last being a precursor of the friedelanyl cation (Scheme 1). This relationship between the baccharenyl and shionanyl cations would make us expect that triterpenes and quinones with shionane skeleton could be isolated from Celastraceae. All this is strongly supported by the fact that the compounds reported in this work and the shionanyl cation have the common intermediate, *D*:*B*-friedobaccharenyl cation.

Compounds **1** and **2** have a novel tetracyclic *D*:*B*-friedobaccharane skeleton, and strongly corroborate the postulated biosynthesis of shionane via *D*:*B*-friedobaccharane. Their presence in species of the Celastraceae family might have chemotaxonomic and phylogenetic importance, and the interesting biological properties showed by similar products encouraged us to pursue the study of these singular products. Baruol (**1**) was tested for biological activity: antimicrobial[12] (MIC > 20 μg/mL), cytotoxic[13] (IC_{50} > 20 μg/mL), and xanthine oxidase (9% of inhibition at 10 μg/mL), β-glucuronidase (57% of inhibition at 10 μg/mL), and β-glucosidase (5% of inhibition at 20 μg/mL) inhibitory effect.[14] Leonal (**2**) could not be assayed as it was unstable.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.07.133. Experimental data and selected NMR spectra of **1** and **2**.

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