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# A novel labdane diterpene from *Acritopappus longifolius*

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ABSTRACT

A novel labdane diterpene was isolated from the plant *Acritopappus longifolius*. The structure of this compound was established by 1D- and 2D-nuclear magnetic resonance spectroscopic techniques and mass spectrometry data. *N*-Methyl-4-hydroxy-*trans*-proline, stigmasterol-3-*O*-β-D-glycoside, and the flavonoids quercetin, luteolin, kaempferol, and rutin were also isolated.



*Acritopappus* (family Asteraceae, tribe Eupatorieae, and subtribe Ageratinae) is a small genus restricted to Eastern Brazil. Sixteen species of this genus are known to date.[1–3] According to King and Robinson, the name *Acritopappus* is due to the irregularity of its pappus form, which accounts for the misplacement of some plant species in this tribe. Recently, some species of *Ageratum*, a genus belonging to this same subtribe, were reclassified by King and Robinson and placed in the *Acritopappus* genus, namely *Acritopappus longifolius, Acritopappus confertus*, and *Acritopappus irwinii*.[1]

Sesquiterpenes, labdane and kolavane diterpenes, triterpenes, steroids, coumarins, acetylenes, flavonoids, and benzofurans derivatives have been reported to occur in this genus.[4–10] Indeed, sesquiterpenes, diterpenes, coumarin, and steroid have been isolated from *A. longifolius* in previous studies.[9,10]

The present study consists of a chemical re-investigation of *A. longifolius*. Along with known compounds, such as flavonoids, steroid, and an unusual amino acid, a novel labdane diterpene has been isolated. This report describes the structural elucidation and a plausible biogenetic pathway for this new compound.

The total plant of *A. longifolius* was dried, and its aerial parts (348 g) were pulverized and macerated with $CH_2Cl_2$ and MeOH at room temperature. The $CH_2Cl_2$ and MeOH extracts were concentrated under reduced pressure, which yielded 52.0 g and 25.1 g of brownish viscous material, respectively. The $CH_2Cl_2$ extract was first partitioned between hexane and MeOH, and later between EtOAc and MeOH. A part of the concentrated EtOAc fraction (9.55 g) was chromatographed over a silica gel column (VLC) and eluted with hexane, hexane/EtOAc mixtures, EtOAc, EtOAc/MeOH mixtures, and finally MeOH. Fractions 5 and 9 furnished the diterpene **1** and stigmasterol-3-*O*-β-D-glycoside,[11] respectively,

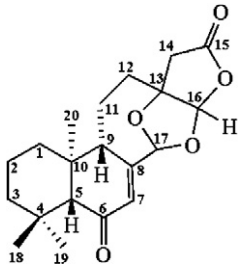

**Figure 1.** Structure **1**.

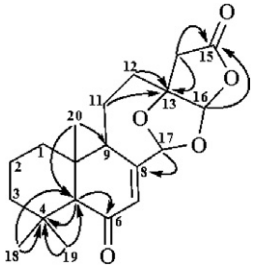

**Figure 2.** Selected HMBC (H→C) correlations for compound **1**.

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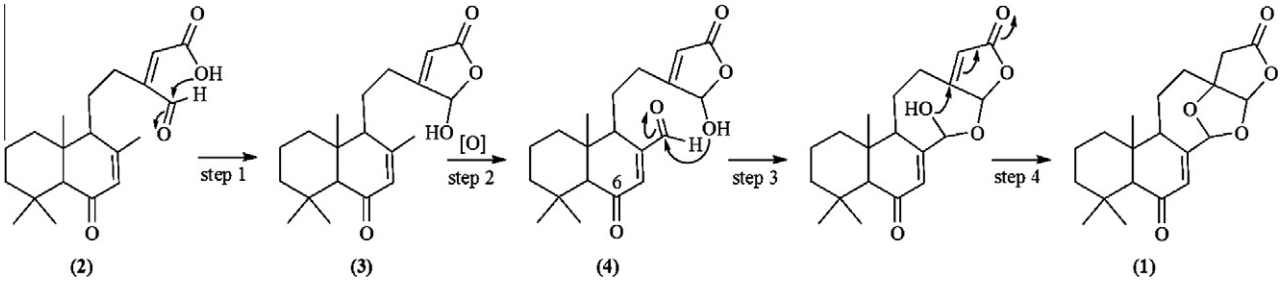


**Figure 3.** Plausible biogenetic pathway to compound **1**.

recrystallized from EtOAc and MeOH. The MeOH extract was partitioned between hexane and MeOH, followed by partitioning in $CH_2Cl_2$/MeOH. The $CH_2Cl_2$ fraction was chromatographed over Sephadex LH-20 column using MeOH as eluent, to give quercetin[12] and kaempferol.[12] The MeOH fraction was chromatographed over a Sephadex LH-20 column using MeOH as eluent, which furnished 16 fractions. Fraction 4 (1.310 g) was separated by CC on silica gel, to yield *N*-methyl-4-hydroxy-*trans*-proline[13] recrystallized from MeOH. Fraction 10 (63.5 mg) contained flavonoids, which were further purified by preparative TLC using $CH_2Cl_2$/MeOH (8:2) as the eluting solvent system, which gave rutin.[14] Fraction 16 furnished luteolin.[12]

Compound **1** $\alpha_D^{25}$ −4.38 (*c* 0.01, $CHCl_3$) was obtained as colorless crystals. Its molecular formula was established as $C_{20}H_{26}O_5$ on the basis of a high-resolution ESITOFMS data at *m*/*z* 345.1722 $[M+H]^+$ (calcd for *m*/*z* 345.1702 $[M+H]^+$). Analysis of the $^1H$ and $^{13}C$ NMR spectroscopic data (Table 1) revealed the presence of 3 methyls ($\delta$ 14.4, 21.3, 32.9); 6 $sp^3$ methylenes ($\delta$ 17.9, 20.8, 38.6, 34.0, 40.3, 42.5); 4 $sp^3$ methines ($\delta$ 52.3, 63.0, 103.8, 109.5); 1 $sp^2$ methine ($\delta$ 129.6); 3 $sp^3$ quaternary carbons ($\delta$ 32.0, 42.7, 89.4); 1 $sp^2$ quaternary carbon ($\delta$ 153.1); and 2 carbonyl carbons ($\delta$ 171.4, 199.9). A review concerning the types of skeletons present in this species and in other species of *Acritopappus* prompted us to consider that **1** was a labdane diterpene.[3–8] The structural elucidation was performed using detailed analyses of $^1H$ and $^{13}C$ NMR spectra and the correlations observed in the HMBC spectrum.

**Table 1**
$^1H$ and $^{13}C$ NMR data and HMBC correlations for **1**

| Posição | $\delta_H$ | $\delta_C$ | HMBC (H→C) |
|---|---|---|---|
| 1a | 1.20; m | 38.6 | C-20 |
| 1b | 1.91; dl; 12.6 | | |
| 2 | 1.5–1.6; m | 17.9 | |
| 3a | 1.39; dl; 11.9 | 42.5 | C-18; C-19 |
| 3b | 1.12; m | | |
| 4 | — | 32.0 | C-18; C-19; C-5 |
| 5 | 2.12; sl | 63.0 | C-18; C-19; C-20 |
| 6 | — | 199.9 | C-5 |
| 7 | 5.88; dl; 2.8 | 129.6 | C-8 |
| 8 | — | 153.1 | C-7; C-17 |
| 9 | 2.48; dl; 11.6 | 52.3 | C-20 |
| 10 | — | 42.7 | C-5; C-20 |
| 11a | 1.5–1.6; m | 20.8 | C-13 |
| 11b | 1.95; m | | |
| 12a | 2.08; m | 34.0 | C-13 |
| 12b | 2.10; m | | |
| 13 | — | 89.4 | C-11; C-12; C-14; C-16 |
| 14a | 2.76; d; 17.7 | 40.3 | C-13; C-15 |
| 14b | 2.95; d; 17.7 | | |
| 15 | — | 171.4 | C-14; C-16 |
| 16 | 5.87; sl | 109.5 | C-15 |
| 17 | 5.91; sl | 103.8 | C-8 |
| 18 | 1.18; s | 21.3 | C-4 |
| 19 | 1.09; s | 32.9 | C-4 |
| 20 | 0.89; s | 14.4 | C-9 |

(Fig. 2). The HMBC spectrum presented correlations of the carbonyl carbon signal at $\delta$ 171.4 (C-15) with both diasterotopic hydrogen signals at $\delta$ 2.76 and $\delta$ 2.95 (H-14a and H-14b) and with the proton signal at $\delta$ 5.87 (H-16). The quaternary carbon at $\delta$ 89.4 (C-13) correlated with the proton signals at $\delta$ 2.76 and 2.95 (H-14a and H-14b), with the proton signal at $\delta$ 5.87 (H-16), and with the proton signals of methylene carbons at $\delta$ 20.8 (C-11) and $\delta$ 34.0 (C-12). The correlations of a proton signal at $\delta$ 5.91 (H-17) with a carbon at $\delta$ 153.1 (C-8) and of a methine carbon at $\delta$ 52.3 (C-9) with methyl proton signal at $\delta$ 0.89 (H-20) were observed, too. The $^{13}C$ chemical shifts of C-17 ($\delta$ 103.8) and C-16 ($\delta$ 109.5) are in agreement with carbons attached by two single bonded oxygens, suggesting that these are acetals. Furthermore, the correlations of the carbonyl carbon C-6 ($\delta$ 199.9) with a methine proton signal at $\delta$ 2.12 (H-5), of a carbon at $\delta$ 63.0 (C-5) with 3 methyls at $\delta$ 1.18 (H-18), $\delta$ 1.09 (H-19) and $\delta$ 0.89 (H-20), and of a quaternary carbon at $\delta$ 32.0 (C-4) with proton signals at $\delta$ 1.18 (H-18), $\delta$ 1.09 (H-19) and 2.12 (H-5) were detected, too. The keto group was assigned at position 6 on the basis of analyses of the correlations obtained from the HMQC and HMBC spectra and the complete assignment of the protons and carbons.

A plausible biogenetic pathway has been proposed for compound **1** herein (Fig. 3). The methyl ester derivative of compound **2** and compound **3** had been isolated from *A. confertus*.[6] A derivative of compound **4** whose difference lay on the absence of a carbonyl group at position 6 had been isolated from *A. longifolius*.[10] On the basis of these compounds previously isolated from this genus and the structure shown in Figure 1, it could be deduced that this new labdane presenting a modified skeleton should have originated from the sequence of cyclization reactions, namely nucleophilic addition (steps 1 and 3), oxidation (step 2), and 1,4-addition (Michael reaction, step 4).

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## Supplementary data

Supplementary data (HRESIMS, $^1H$ and 2D NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.11.040.

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