

0031-9422(95)00499-8

# A DIMERIC SINAPALDEHYDE GLUCOSIDE FROM ILEX ROTUNDA

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## (Received in revised form 5 June 1995)

Key Word Index—*Ilex rotunda*; Aquifoliaceae; bark; ilexrotunin; dimeric sinapaldehyde glucoside; rotundanonic acid.

Abstract—Investigation of the Chinese medicinal plant *Ilex rotunda*, afforded, in addition to known compounds, a dimeric sinapaldehyde glucoside, ilexrotunin, and rotundanonic acid. The structures were elucidated by spectroscopic methods.

## INTRODUCTION

Ilex species are distributed widely in China and some have been used extensively in folk medicine, such as *I.* pubescens, *I. chinensis* and *I. asperlla*. A decoction of the roots of *I. pubescens* has shown coronary vasodilation in animal tests and is used for the treatment of coronary disease, myocardial infarction and Burger's disease [1]. Ilex chinensis is taken as an antibacterial and is used as a remedy for pneumonia, tonsillitis, urinary tract infections and the common cold, and also shows coronary vasodilation activity [2]. Leaves of *I. asperlla* are applied to snake-bites and used as an antibiotic [3]. Ilex rotunda is a commonly known antipyretic and antidote for the treatment of the common cold, tonsillitis, stomach and intestinal ulcers [4].

Chemical investigations of *llex* species have shown that they contain rotundic acid (1) [5], pedunculoside (2) [6], syringin (4) [7, 8], rotungenic acid, rotundioic acid and other known triterpenoid compounds [9]. In our study, we examined the chemical components of the bark of *I. rotunda*. In addition to eight known compounds, rotundanonic acid (7) and the dimeric sinapaldehyde glucoside, ilexrotunin (8), were isolated.

## **RESULTS AND DISCUSSION**

The ethanol extract of the bark of *I. rotunda* was partitioned between petrol and methanol. The methanol layer was further treated with acetone. The acetone-soluble fraction was repeatedly chromatographed to afford rotundic acid (1), pedunculoside (2), syringaldehyde (3), syringin (4), sinapaldehyde (5), sinapaldehyde glucoside (6), 3-O-acetyloleanolic acid, stearic acid, rotundanonic acid (7) and ilexrotunin (8).

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Rotundanonic acid (7) showed a  $[M]^+$  at m/z 486.3357 suggesting the molecular formula  $C_{30}H_{46}O_5$ . Its spectral data showed unconjugated keto absorption at 285 nm in UV and 3480(OH), 1700(CO), 1450(double bond) cm<sup>-1</sup> in IR. In the <sup>1</sup>H NMR, signals at  $\delta 0.84 s$ , 0.87 s, 1.0 s, 1.36 s and 0.93 d, together with six methyl signals were observed typical of ursene-type triterpenes. In the downfield region, trisubstituted olefinic protons at  $\delta 5.28 t$  were also observed.

The ring system and substitution of 7 was obtained from its EI-mass spectrum in accordance with the known fragmentation patterns of triterpenes. There were two characteristic peaks at m/z 264 [a]<sup>+</sup> and 222 [b]<sup>+</sup>, ascribed to retro-Diels-Alder cleavage fragments commonly found in spectra of olean-12-ene or urs-12-ene derivatives possessing one hydroxyl group and one keto group on ring A/B and one hydroxyl group and one carboxyl group on ring D/E [5]. Further losses of  $H_2O_1$ , COOH and  $CH_2OH$  from  $[a]^+$  or  $[b]^+$  were observed at 246  $[a - H_2O]^+$ , 219  $[b - COOH]^+$  and 191  $[b - CH_2OH]^+$ . The peak at m/z 219  $[a - COOH]^+$ was four times more intense than  $[a]^+$ . This indicates that the COOH must be present at C-17 [10, 11]. The <sup>1</sup>HNMR spectrum of 7 exhibited a methyl signal at  $\delta 0.93 d$  and a downfield methyl signal at  $\delta 1.36 s$ , indicating that a hydroxyl group may be connected to C-19 or C-20. The <sup>1</sup>H NMR spectrum also showed a H-18 singlet at  $\delta 2.51$ . This evidence indicated that the hydroxyl group is located at C-19. The stereochemistry of the C/D ring in 7 was established as cis-fused by NOE difference spectroscopy; saturation of the H-12 olefinic proton gave NOE with H-18 (8.37%) and irradiation of H-18 gave NOE with H-12 (7.25%). It is thus identical with rotundic acid (1) at the C/D ring junction. The <sup>1</sup>H NMR of 7 also showed a pair of AB-system protons at  $\delta$  3.41 and 3.32, with coupling constants of 10.4 Hz and 2.42 Hz ddd and a signal at  $\delta 2.35 \, ddd$  corresponding the two  $\alpha$ -protons of a keto carbonyl, but the H-3 proton found in rotundic acid was not present. Rotundic acid (1) could be oxidized





with Jones reagent in acetone to give 7. Therefore, the structure of rotundanonic acid is  $19\alpha$ -23-dihydroxy-3-keto- $\Delta^{12}$ -ursane-28-oic acid (7).

Dimeric sinapaldehyde glucoside (8) was obtained as yellow needles,  $C_{30}H_{44}O_{18}$ . It showed UV absorption at 235 nm and 315, and IR absorption at 3400(OH), 1590 and 1460 (Ar) cm<sup>-1</sup>. The <sup>1</sup>H NMR spectrum exhibited a signal at  $\delta 6.90 \ s$  (2H) for two equivalent aromatic protons,  $\delta 6.85 \ d$  ( $J = 16.0 \ Hz$ ) and  $\delta 6.40 \ (J = 16.0 \ and 4.6 \ Hz)$  for a pair of *trans*-olefinic protons, and one at  $\delta 6.40$  coupled with 5.86 d ( $J = 4.6 \ Hz$ ). These signals indicate the presence of a



moiety in the molecule. The <sup>1</sup>HNMR spectrum also showed signals at  $\delta 3.38$  and 3.75, for two methoxyl groups and  $\delta 5.09 d$  (J = 5.7 Hz, H-1'), 4.35 m (2H, H-6') and 3.38-4.35 corresponding to glucosyl protons. After enzyme hydrolysis of **8**, glucose and sinapaldehyde were detected by TLC.

Comparison between the spectra of **8** and sinapaldehyde glucoside **6**, revealed that the  $v_{CHO}$  and  $\delta_{CHO}$  had disappeared in the IR and <sup>1</sup>H NMR spectra of **8** and that the signal of the glucosyl 6'-protons of **6** ( $\delta$ 4.50) had shifted upfield to  $\delta$ 4.35. Based on the above data, **8** was elucidated as a hemiacetal of sinapalderdehyde glucoside, which may be condensed by hemiacetal formation. Dreiding stereomolecular models indicated that it is impossible for **6** to form an intramolecular condensation product with a *trans*-linked double bond.

Veith et al. [12] have reported ion-formation FD-MS for measuring the  $[M]^+$  of nonvolatile and polar organic compounds and successfully determined the molecular formula of loroglossin  $C_{34}H_{46}O_{18}$ , a diglucoside from the Orchidaceae [13]. Compound 8 formed an ion-cluster at m/z 763  $[M + Na]^+$  and 741  $[M + 1]^+$ . Therefore, we deduced that 8 is a dimeric sinapaldehyde glucoside. The bis-hemiacetal structure of 8 can be regarded as a product of intermolecular addition between two molecules of 6. The formation of the hemiacetal is reversible under acidic conditions. We found that 8 partially decomposed in acid solution to form 6, which could be detected by TLC.

#### EXPERIMENTAL

General. Mps: uncorr. IR: KBr discs; <sup>1</sup>H NMR: Bruker AM-400.

Plant material. Ilex rotunda Thunb. was collected in Guangxi province (southern China) in the summer and identified by Prof. Shu-quan Zhong. A voucher specimen is deposited at the Guangxi Institute of Botany.

Extraction and isolation. Air-dried plant material was extracted with EtOH and the resulting extract was distributed between petrol and MeOH. The MeOH fr. was dissolved in Me<sub>2</sub>CO and the Me<sub>2</sub>CO-sol. portion chromatographed on silica gel with petrol and Et<sub>2</sub>O mixts of increasing polarity and then further purified by rechromatography and separated by TLC. This afforded rotundic acid (1) 10 g, pedunculoside (2) 5 g, syrigin (4) 5 g, rotundanonic acid (7) 200 mg, sinapaldehyde (5) 50 mg, sinapaldehyde glucoside (6) 50 mg, 3-O-acetyl olealic acid 100 mg, syringaldehyde (3) 50 mg, stearic acid 10 mg and ilexrotunin (8) 10 mg.

Rotundanonic acid (7). Mp 215–217°.  $[\alpha]_D + 101^\circ$  (c 0.20; Me<sub>2</sub>CO). HRMS: m/z 486.3357 [M]<sup>+</sup> C<sub>30</sub>H<sub>46</sub>O<sub>5</sub> (calc. 486.3354). EIMS m/z (rel. int.): 486 [M]<sup>+</sup> (1), 440 [M - COOH]<sup>+</sup> (8), 410 [M - HCOOH - HCHO]<sup>+</sup> (29), 264 [a]<sup>+</sup> (2), 246 [a - H<sub>2</sub>O]<sup>+</sup> (1), 222 [b]<sup>+</sup> (2), 218 [a - COOH]<sup>+</sup> (1) 191 [b - CH<sub>2</sub>OH]<sup>+</sup> (7). IR: (KBr) 3480 (OH), 1700 (CO), 1650 (C=C). UV: (MeOH) 285 nm (log  $\varepsilon$  1.79). <sup>1</sup>H NMR (acetone- $d_6$ ):  $\delta$ 0.84, 0.87, 1.00, 1.19, 1.36 (s, each 3H), 0.93 d (3H, J = 6.5 Hz), 2.35 ddd (H-2), 2.42 ddd (H-2'), 2.51 s (H-18), 3.41 d (2H, J = 10.4, H-23), 5.28 t (J = 3.4, H-12).

Oxidation of rotundic acid. To a soln of 20 mg rotundic acid (1) in 10 ml Me<sub>2</sub>CO, 0.1 ml Jones reagent (5 g CrO<sub>3</sub> in 5 ml H<sub>2</sub>SO<sub>4</sub> dild to 20 ml) was added and oxidized in the usual manner to afford to 10 mg of 7. Mp 215–217°, IR, TLC same as natural product 7.

*llexrotunin* 8. Mp 151–152°. FDMS: m/z (rel. int.) 763 [M + Na]<sup>+</sup> C<sub>34</sub>H<sub>44</sub>O<sub>18</sub> (67), 741 [M + H]<sup>+</sup> (9), 393 [C<sub>17</sub>H<sub>22</sub>O<sub>9</sub> + Na]<sup>+</sup> (100), 231 (18), 99 (10). IR: (KBr) 3400 (OH), 1590, 1460 (Ar). <sup>1</sup>H NMR (C<sub>5</sub>D<sub>5</sub>N):  $\delta$ 3.38-4.35 (protons of glucosyl moiety), 4.35 m (4H, H-6'), 5.90 d (2H, J = 5.7 Hz, H-1'), 5.85 d (2H, J = 4.6 Hz), 6.40 dd (2H, J = 16.0, 4.6 Hz), 6.85 (2H, J = 16.0 Hz), 6.90 s (4H, Ar-H), 3.38 s (6H, OCH<sub>3</sub>), 3.75 s (6H, OCH<sub>3</sub>).

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