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A DIMERIC SINAPALDEHYDE GLUCOSIDE FROM *ILEX ROTUNDA*

DONG-XU WEN and ZHONG-LIANG CHEN*

Shanghai Institute of Materia Medica, Academia Sinica, Yue-yang Road 319, Shanghai 200031, People's Republic of China

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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 *Ilex* 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.

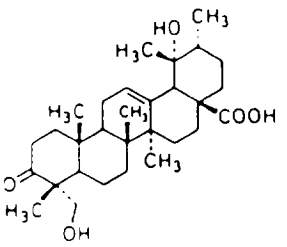
RESULTS AND DISCUSSION

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**).

*Author to whom correspondence should be addressed.

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^{-1} in IR. In the ^1H NMR, signals at δ0.84 *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 δ5.28 *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 $[a]^+$ and 222 $[b]^+$, 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, 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 ^1H NMR spectrum of **7** exhibited a methyl signal at δ0.93 *d* and a downfield methyl signal at δ1.36 *s*, indicating that a hydroxyl group may be connected to C-19 or C-20. The ^1H NMR spectrum also showed a H-18 singlet at δ2.51. 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 ^1H NMR of **7** also showed a pair of AB-system protons at δ3.41 and 3.32, with coupling constants of 10.4 Hz and 2.42 Hz *ddd* and a signal at δ2.35 *ddd* corresponding the two α-protons of a keto carbonyl, but the H-3 proton found in rotundic acid was not present. Rotundic acid (**1**) could be oxidized



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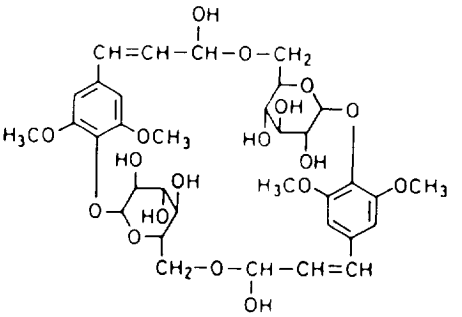

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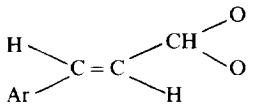
with Jones reagent in acetone to give **7**. Therefore, the structure of rotundanonic acid is 19α-23-dihydroxy-3-keto-Δ^{12}-ursane-28-oic acid (**7**).

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^{-1}. The ^1H NMR spectrum exhibited a signal at δ6.90 *s* (2H) for two equivalent aromatic protons, δ6.85 *d* (J = 16.0 Hz) and δ6.40 (J = 16.0 and 4.6 Hz) for a pair of *trans*-olefinic protons, and one at δ6.40 coupled with 5.86 *d* (J = 4.6 Hz). These signals indicate the presence of a



moiety in the molecule. The ^1H NMR spectrum also showed signals at δ3.38 and 3.75, for two methoxyl groups and δ5.09 *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 ν_{CHO} and δ_{CHO} had disappeared in the IR and ^1H NMR spectra of **8** and that the signal of the glucosyl 6′-protons of **6** (δ4.50) had shifted upfield to δ4.35. 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; ^1H 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_2CO and the Me_2CO-sol. portion chromatographed on silica gel with petrol and Et_2O 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 oleanolic 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° (*c* 0.20; Me_2CO). HRMS: *m/z* 486.3357 $[M]^+$ $C_{30}H_{46}O_5$ (calc. 486.3354). EIMS *m/z* (rel. int.): 486 $[M]^+$ (1), 440 $[M - COOH]^+$ (8), 410 $[M - HCOOH - HCHO]^+$ (29), 264 $[a]^+$ (2), 246 $[a - H_2O]^+$ (1), 222 $[b]^+$ (2), 218 $[a - COOH]^+$ (1) 191 $[b - CH_2OH]^+$ (7). IR: (KBr) 3480 (OH), 1700 (CO), 1650 (C=C). UV: (MeOH) 285 nm (log ε 1.79). ^1H NMR (acetone-d_6): δ0.84, 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_2CO, 0.1 ml Jones reagent (5 g CrO_3 in 5 ml H_2SO_4 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**.

Ilexrotunin **8**. Mp 151–152°. FDMS: *m/z* (rel. int.) 763 $[M + Na]^+$ $C_{34}H_{44}O_{18}$ (67), 741 $[M + H]^+$ (9), 393 $[C_{17}H_{22}O_9 + Na]^+$ (100), 231 (18), 99 (10). IR: (KBr) 3400 (OH), 1590, 1460 (Ar). ^1H NMR (C_5D_5N):

δ3.38–4.35 (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_3), 3.75 *s* (6H, OCH_3).

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