Communications to the Editor

[Chem. Pharm. Bull. 36(3)1237—1240(1988)]

NEW 5β-H LIMONOIDS FROM EVODIA RUTAECARPA BENTHAM

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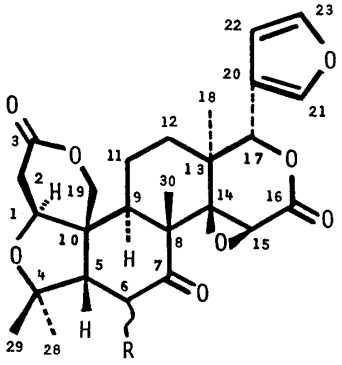
Two new 5β-H limonoids, 6α-acetoxy-5-epilimonin (1) and 6β-acetoxy-5-epilimonin (2), were isolated from the fruit of Evodia rutaecarpa BENTHAM (Rutaceae), together with limonin (3) and evodol (4), and their structures were determined based on the ^{1}H- and ^{13}C-NMR spectral data.

KEYWORDS —— limonoid; Evodia rutaecarpa; Rutaceae; 6α-acetoxy-5-epilimonin; 6β-acetoxy-5-epilimonin; limonin; evodol; NMR

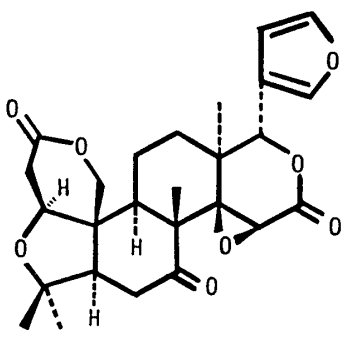
In the course of our study on the biologically active compounds of Rutaceous plants, we have isolated two new minor limonoids, 6α-acetoxy-5-epilimonin (1) and 6β-acetoxy-5-epilimonin (2), from the fruit of Evodia rutaecarpa BENTHAM and found that they have an uncommon 5β-H configuration.[1] Recently, Nakatani et al.[2] have reported the isolation and structure determination of graucin A, a new bitter limonoid having the 5β-H configuration, from the roots of E. grauca MIQ. This report prompted us to publish the results of our structure elucidation of 1 and 2.

The MeOH extract of Evodiae fruit was separated into the AcOEt-soluble part and the H_2O-soluble one. The former was further fractionated by a combination of silica gel chromatography and HPLC to give new limonoids, 6α-acetoxy-5-epilimonin (1) and 6β-acetoxy-5-epilimonin (2), along with known limonoids limonin (3)[3] and evodol (4).[4]

6α-Acetoxy-5-epilimonin (1), $C_{28}H_{32}O_{10}$, was obtained as colorless rods (from MeOH), mp 256-258°C, $[\alpha]_D^{20}$ -93.5° ($CHCl_3$), UV λ_{max}^{MeOH} nm: 208 (log ε=3.76), IR ν_{max}^{KBr} cm^{-1}: 1770



1: R = α-OAc
2: R = β-OAc



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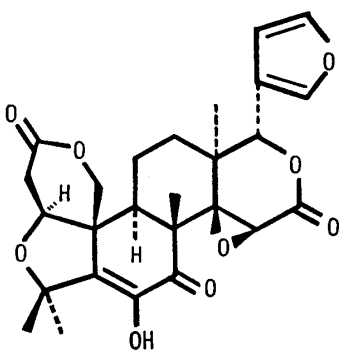

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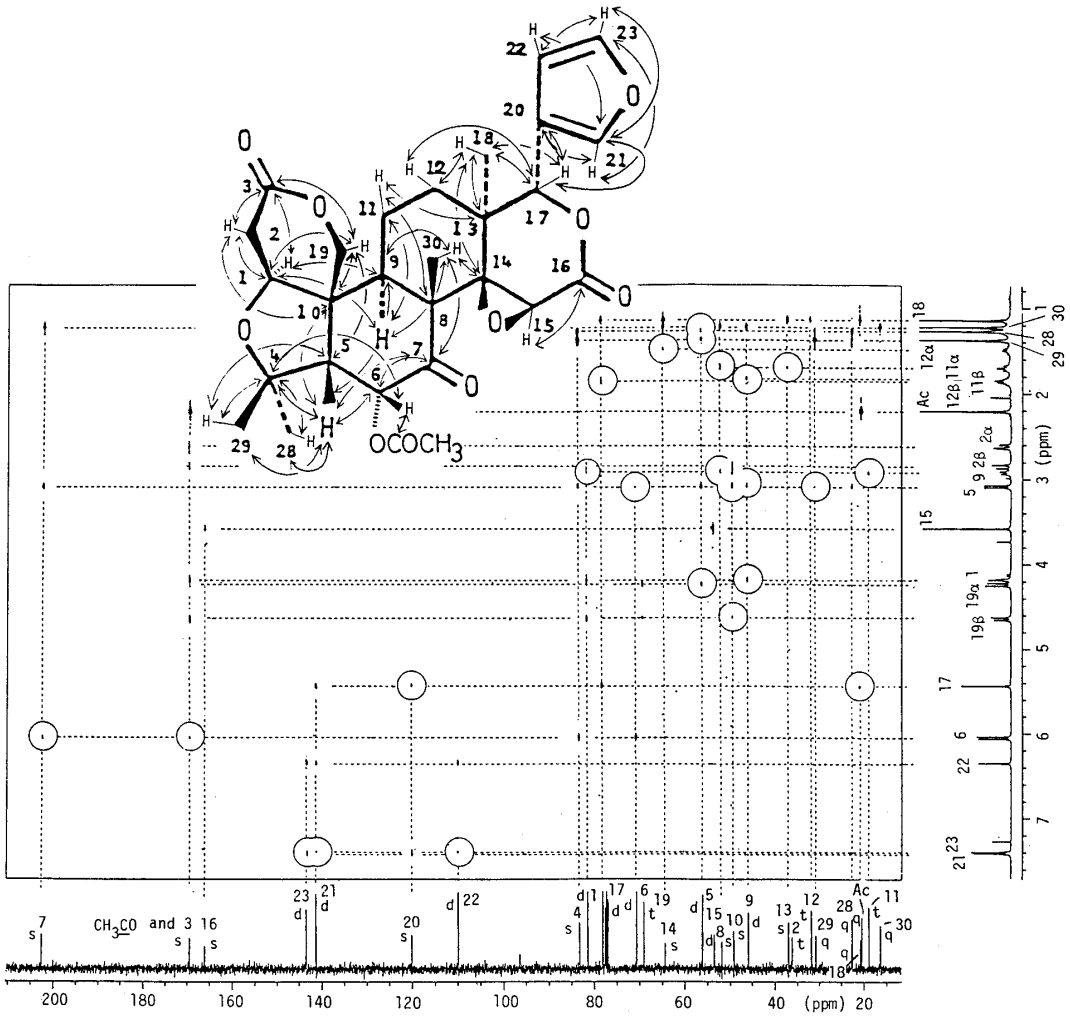

Fig. 1. ^{1}H-^{13}C Long-Range Correlation Spectrum of 6α-Acetoxy-5-epilimonin (1) in $CDCl_3$ (Sample: 11 mg, 12 h run, J_{CH} = 10 Hz)

and 1750, CD: $[\theta]_{287}^{MeOH}$-4000, $[\theta]_{227}^{MeOH}$-19000 (c=0.0009). The EIMS of 1 showed the M^+ peak at m/z 528 and fragment ion peaks at m/z 513 $(M-CH_3)^+$, 453 $(M-CH_3-CH_3COOH)^+$. Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of 1 in ^{1}H-decoupling experiments and ^{1}H-^{13}C COSY suggested the presence of four tert-methyl groups (δH 1.14, 1.22, 1.27, and 1.37), a cyclohexanone, an acetoxyl group (δH 2.21), a β-substituted furan (δH 6.35, 7.40, and 7.42), a lactone (δH 2.61, 2.85, 4.23, and 4.64), and an epoxy-lactone (δH 3.58 and 5.44). This indicates that 1 may be a compound closely related to limonin (see also Table I).

Treatment of 1 with $KHCO_3$ in MeOH gave a diosphenol (4), which was identical with evodol (4) by the ^{1}H-NMR comparison.

Next, we applied the ^{1}H-^{13}C long-range COSY to 1 in order to confirm the structure. As shown in Fig. 1, the carbon signal at δ 169.2 ($CH_3\underline{C}OO$) showed long-range correlation with the ^{1}H-signal at δ 6.05 (6-H), while the signal at δ 202.5 (7-C) with those at δ 1.22 (30-H_3), 3.08 (5-H), and 6.05 (6-H). Also, the methyl carbon signals at δ 20.7, 22.6, and 30.7 are correlated with the ^{1}H-signals at δ 5.44 (17-H), at δ 1.37 (29-H_3) and 3.08 (5-H), and at δ 1.27 (28-H_3) and 3.08 (5-H), respectively.

Table I. ^{13}C-NMR Spectral Data of 6α-Acetoxy-5-epilimonin (1), 6β-Acetoxy-5-epilimonin (2), and Limonin (3) (δ in $CDCl_3$)

Compd / ^{13}C	1	2	3		1	2	3
1	81.2 d	78.3 d	79.1 d	15	53.3 d	55.9 d	53.9 d
2	36.0 t	35.1 t	35.7 t	16	165.9 s	165.9 s	166.6 s
3	169.3 s	169.5 s	169.1 s	17	77.9 d	77.9 d	77.8 d
4	83.0 s	82.5 s	80.3 s	18	20.7 q	20.7 q	20.7 q
5	55.9 d	56.5 d	60.5 d	19	68.9 t	70.7 t	65.4 t
6	70.4 d	73.9 d	36.4 t	20	119.9 s	119.9 s	120.0 s
7	202.5 s	202.9 s	206.2 s	21	141.1 d	141.3 d	141.1 d
8	51.7 s	48.1 s	51.3 s	22	109.7 d	109.3 d	109.7 d
9	45.8 d	39.8 d	48.1 d	23	143.3 d	143.4 d	143.2 d
10	49.1 s	48.6 s	45.9 s	28	22.6 q	22.1 q	30.2 q
11	18.9 t	17.1 t	18.9 t	29	30.7 q	29.7 q	21.4 q
12	31.8 t	26.9 t	30.8 t	30	16.3 q	17.0 q	17.6 q
13	36.8 s	38.9 s	37.9 s	$\underline{C}H_3COO$	20.4 q	20.3 q	
14	64.2 s	68.3 s	65.7 s	$CH_3\underline{C}OO$	169.2 s	169.8 s	

The many carbon signals were determined by the off-resonance and are indicated as s, d, t, and q. Assignments were done by the use of ^1H-^{13}C COSY and long-range COSY.

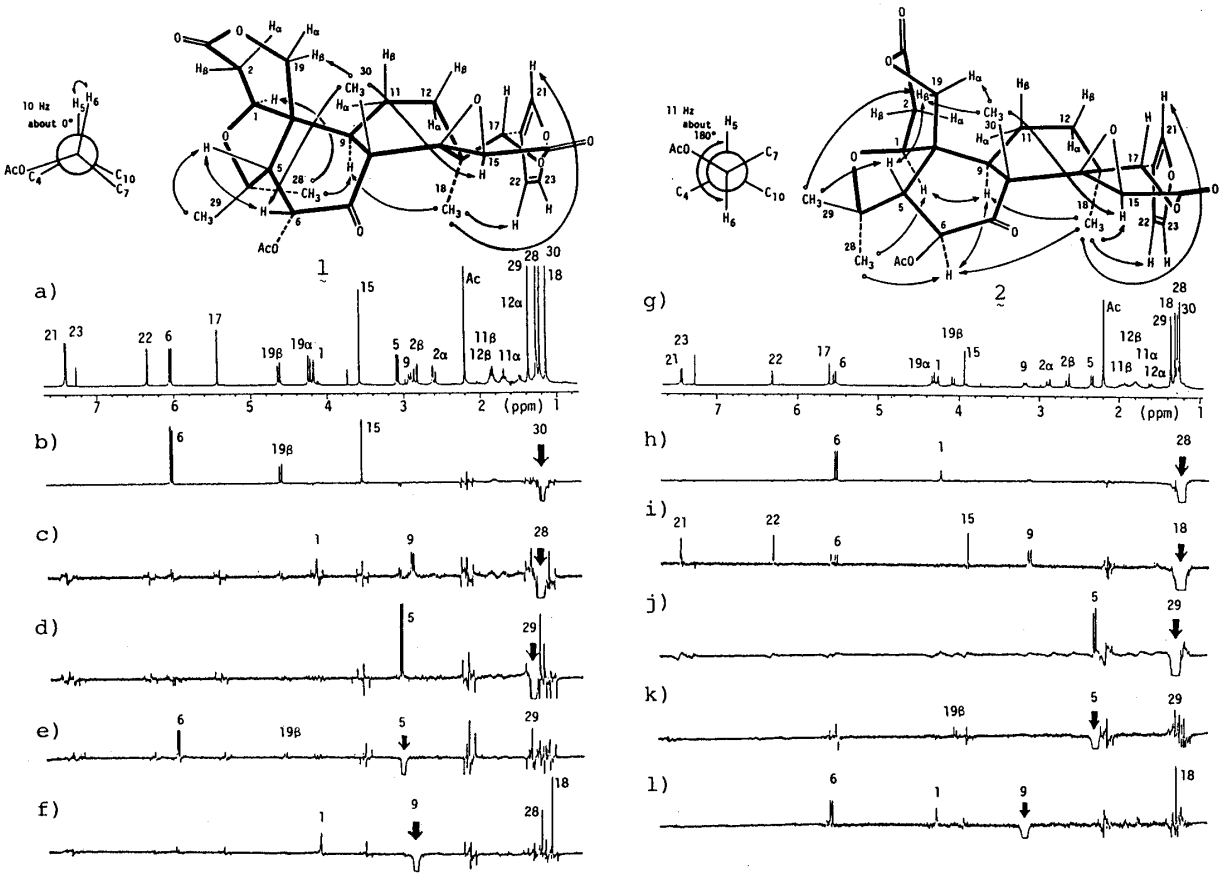

Fig. 2. Normal ^1H-NMR and NOE Difference Spectra of 6α-Acetoxy-5-epilimonin (1) (a - f) and 6β-Acetoxy-5-epilimonin (2) (g - l) in $CDCl_3$

Therefore, those carbons at δ 20.7, 22.6, and 30.7 in the spectrum of 1 were assigned to 18-, 28-,[5] and 29-C,[5] respectively. Some other significant long-range correlations observed are also shown by arrows in Fig. 1.

From the above observation together with the ^{1}H-NMR data it appears that 1 is a limonoid having 6-acetoxyl group.

The relative stereochemistry of 1 was determined on the basis of the coupling constants of each proton and the results of NOE experiments. Irradiation at the 30-H_3 and 28-H_3 enhanced the signal intensity of the 6-, 15-, and 19β-protons and the 1- and 9-protons, respectively, and irradiation at the 29-H_3 and 5-H enhanced the signal intensity of the 5-proton and the 6- and 29-protons, respectively(Fig.2,a-e). Also there were NOEs between the 9-H and the 18-H_3, 28-H_3, and 1-H. In view of the coupling constant of 6-H (10 Hz), it appears that the B-ring has a half-chair conformation in which the dihedral angle between 5-H and 6-H is almost 0° (Fig. 2).

6β-Acetoxy-5-epilimonin (2),[6] $C_{28}H_{32}O_{10}$, was obtained as colorless crystals (from MeOH), mp 229-231°C, $[\alpha]_D^{20}$ +41° ($CHCl_3$), UV λ_{max}^{MeOH} nm: 206 (logε=3.78), IR ν_{max}^{KBr} cm^{-1}: 1770 and 1750, CD: $[\theta]_{303}^{MeOH}$ +11000, $[\theta]_{295}^{MeOH}$ +12000, $[\theta]_{230}^{MeOH}$ -11500 (c= 0.0006). This compound was considered to be the 6-epimer of 1 from comparisons of the ^{1}H- and ^{13}C-NMR spectral data with those of 1. This was supported by the NOE experiments, in which NOEs appeared between the 28-H_3 and the 6-H as well as between the 29-H_3 and the 5-H, as shown in Fig. 2 (h and j). The coupling constant of 6-H (11 Hz) in 2 suggested that the B-ring has a boat conformation in which the dihedral angle between 5-H and 6-H is about 180° (Fig. 2).

It should be noted that the 5β-H limonoids are rare in nature and only a few 5β-H-6-oxo compounds have been reported so far.[2,7] Our present result provided the first example of 5β-H-6-acetoxy limonoids, which are not enolizable at the 5-position. They are of interest from the biogenetic view point.

REFERENCES AND NOTES

1) This work was presented at the 34th Annual Meeting of Japanese Society of Pharmacognosy of Japan, Osaka, October 1987, Abstr., p. 134.

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4) Y. Hirose, *Chem. Pharm. Bull.*, 11, 535 (1963).

5) Assignments were confirmed by the NOE experiments.

6) 2: ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H,s, 30-H_3), 1.28 (3H,s, 28-H_3), 1.31 (3H,s, 18-H_3), 1.36 (3H,s, 29-H_3), 1.60 (1H,ddd, J = 15, 9, 2 Hz, 12-Hα), 1.78 (1H,dddd, J = 15, 9, 4.5, 2 Hz, 11-Hα), 1.83 (1H,ddd, J = 15, 6, 2 Hz, 12-Hβ), 1.94 (1H,dddd, J = 15, 12.5, 6, 2 Hz, 11-Hβ), 2.21 (3H, s, CH_3CO), 2.34 (1H,d, J = 11 Hz, 5-H), 2.64 (1H, dd, J = 16, 3 Hz, 2-Hβ), 2.89 (1H,dd, J = 16, 3 Hz, 2-Hα), 3.18 (1H,dd, J = 12.5, 4.5 Hz, 9-H), 3.93 (1H,s, 15-H), 4.07 (1H,d, J = 12 Hz, 19-Hβ), 4.26 (1H, t, J = 3 Hz, 1-H), 4.32 (1H, d, J = 12 Hz, 19-Hα), 5.54 (1H,d, J = 11 Hz, 6-H), 5.61 (1H,s, 17-H), 6.31 (1H,dd, J = 1.8, 0.8 Hz, 22-H), 7.43 (1H,t, J = 1.8 Hz, 23-H), 7.45 (1H, dd, J = 1.8, 0.8 Hz, 21-H).

7) R. D. Benett and S. Hasegawa, *Tetrahedron*, 37, 17 (1981).

(Received December 25, 1987)