Spirosendan, a Novel Spiro Limonoid from *Melia Toosendan*

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Spirosendan, a new skeletal limonoid possessing a spiro-structure, was isolated from the root bark of *Melia toosendan* (Meliaceae). The structure is proposed on the basis of extending spectroscopic means.

Limonoids from *Melia* species are attracting considerable interest, because of a variety of the structures and their biological activities.[1] In a series of our experiments on Meliaceae plants, several types of limonoids were isolated as insect antifeedant from *Trichilia roka*[2] and *Melia azedarach*.[3] *Melia toosendan*, closely related to *M. azedarach*, is a large and native tree to China, in which an extract of the bark is used as an anthelmintic. We have already reported the isolation of eighteen new limonoids as antifeeding constituents from the stem and root bark.[3] In the continuous study, we have isolated a new novel limonoid, named spirosendan (**1**), possessing a new skeleton with a spiro-structure from the ether extract of the air-dried root bark (1.5 kg). In this paper, we report structural elucidation of the new spiro compound **1**.

Compound **1** (2.5 mg),[5] $[\alpha]_D$ -2° (c 0.07), was shown, by accurate mass measurement (HRFAB-MS: m/z 643.3129 $[M+1]^+$; Δ +1.1 mmu), to have the molecular formula $C_{35}H_{46}O_{11}$ (13 unsaturations). The UV maximum at 219 nm and IR absorption at 3550-3250, 1735, 1705, 1640 and 1618 cm^{-1} showed the presence of carbon-carbon double bonds, hydroxyl groups and carbonyl (ester) moieties. From its ^1H and ^{13}C NMR data, it was evident that six of the elements of unsaturation were present as double bonds: three carbon-carbon (two as a furan ring and the other as a part of a conjugated ester) and three CO (esters). Thus, the molecule is heptacyclic. It was also clear from the NMR data (Table 1) that **1** contained 7 CH_3 (three tertiary, two olefinic and two acetyl), 5 CH_2 , 12 CH (four olefinic), 11 carbons (two olefinic) not bonded to hydrogen, and two protons due to OH groups. Furthermore, the spectra showed the presence of a β-furyl moiety, and two acetyl and one tigloyl groups.

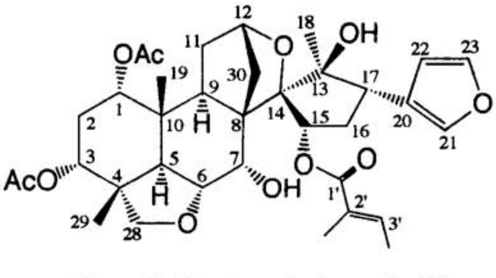

Figure 1. Structure of spirosendan (**1**).

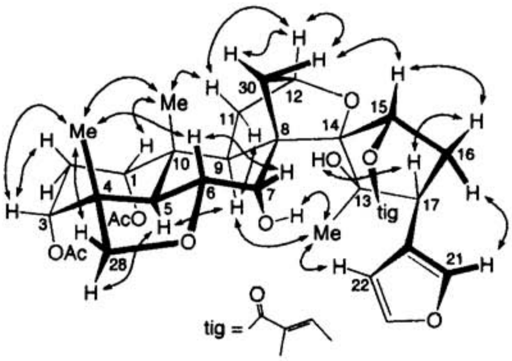

Figure 2. Selected NOE correlation in **1**.

After assignment of all protons to their directly bonded carbon atoms by the HSQC spectrum (Table 1), it was possible to deduce two major molecular fragments from the ^1H-^1H COSY spectrum, decouplings, NOE measurements (Figure 2) and HMBC spectrum. Thus, H-7β, sharpened by the addition of D_2O, coupled with H-6β attached to a carbon bearing an oxygen function, which in turn showed a coupling with H-5 and an NOE correlation with H_3-29. The 4β-Me (29) signal showed NOEs with the signals of H-3β, 28β (one of methylene AB quartet), and H_3-19, which also showed an NOE with the H-1β signal. Further W-shape long range couplings of the 4β- (29) methyl signal with the 28α proton signal and the 10β- (19) methyl signal with the H-9 signal clearly defined the first molecular fragment, C-1 to C-10 in the A/B ring system including 10β-/4β-Me and 4α-methyleneoxy bridge to C-6. These assignments were unambiguously confirmed from the HMBC correlation (Figure 3). Similarly, couplings of H-17 with H_2-16 and H_2-16 with H-15, as well as a W-shape long range coupling between H-17 and H_3-18, characterized a second fragment of the molecule, C-15 to C-17 of the D-ring in limonoid skeleton. The fact that a β-furan ring and a tigloyloxy group were attached to C-17 and C-15 was also confirmed by the HMBC spectrum.

Three tertiary methyls at 4β (29), 10β (19) and 13α (18) and one methylene group at 4α (28) in the basic limonoid skeleton were easily confirmed, and that the remaining methyl group to be at C-8 was changed to a methylene group directly attached to the 12-methine carbon in **1**, was confirmed by the

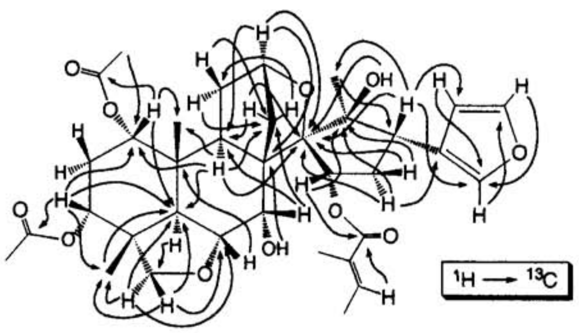

Figure 3. Selected HMBC correlation in **1**.

HMBC spectrum. Actually the ^{1}H-^{1}H couplings from these proton resonance could not be interpreted unambiguously due to signal overlapping. The HMBC spectrum made with **1** permitted these subjects to be solved, that is, the tendency of their connections, especially the C_{30}-C_{12} carbon linkage. Thus long-range ^{1}H-^{13}C couplings from H-7 and 9 to C-30 and between H_2-30 and C-8, 9, 11, 12 and 14, as well as between H_3-18 and C-14 and between H-12 and C-14, clearly established the C-12 to C-13 fragment through C-30, 8 and 14. Similarly the C-13 to C-17 fragment was also established.

The relative configuration assignment of the five chiral centers of the right half of **1** was established from the NOESY experiment. NOE correlation of the signal of $H_{pro\text{-}R}$-30 with the signals of H-12 and H-15β, suggested a spatial proximity of the H-30 proton to H-15β. In addition, the H-15β signal showed a cross peak with the signal of H-16β, which in turn showed an NOE with the signal of H-17β, suggesting α- orientation of the furan ring. On the other hand, the NOE correlation of H_3-18 at C-13α with H-9α, 7-OH and H-22 suggested that these protons were present in the same α side of the molecule and then another hydroxyl group at C-13 was β, which was also confirmed from an NOE with the H-17β signal. Consequently, the chirality of the C-14 spiro-center is *R*.

Table 1. ^{13}C and ^{1}H NMR data for compound **1**

atom	δ_C	δ_H	atom	δ_C	δ_H
1	72.2 d	4.64 t	17	45.2 d	2.84 dd
		(2.9)			(12.8, 7.0)
2α	28.4 t	2.05 br dt	18	20.7 q	1.29 s
		(4.0, 2.9)	19	16.9 q	0.91 s
β		2.06 br dt	20	123.9 s	
		(4.0, 2.9)	21	142.7 d	7.17 m
3	71.8 d	4.84 t	22	111.4 d	6.36 d
		(2.8)			(1.3)
4	43.0 s		23	139.9 d	7.30 dd
5	39.4 d	2.61 d			(1.7, 1.3)
		(12.5)	28α	79.1 t	3.59 br d
6	74.9 d	3.87 dd			(7.5)
		(12.5, 3.9)	β		3.56 d
7	67.3 d	4.42 d	29	20.8 q	1.19 br s
		(3.9)	$30_{pro\text{-}R}$	42.6 t	1.85 m
8	60.3 s		*pro-S*		1.47 m
9	35.9 d	3.02 dd	1-Ac	21.3 q	1.96 s
		(9.7, 5.2)		170.7 s	
10	39.3 s		3-Ac	21.9 q	2.03 s
11α	34.0 t	1.55 m		170.7 s	
β		1.65 br dd	tigloyl		
		(13.5, 5.2)	1'	167.6 s	
12	76.6 d	5.35 br s	2'	129.3 s	
13	81.6 s		3'	138.5 d	6.90 qq
14	96.7 s				(7.2, 1.1)
15	80.7 d	5.37 t	2'-Me	12.5 q	1.82 dq
		(7.7)			(1.4, 1.1)
16α	32.6 t	1.50 m	3'-Me	15.0 q	1.77 dq
β		2.30 ddd			(7.2, 1.1)
		(12.8, 12.0, 7.7)	7-OH		2.58 s
			13-OH		3.10 s

Measured in $CDCl_3$ at 125 and 500 MHz.

Although some spiro limonoids have been reported,[6,7] this is, to the best of our knowledge, the first report of the occurrence of this type of limonoids from a Meliaceae plant. The antifeedant activity of spirosendan (**1**) was tested by a conventional leaf disk method[8] against the third-instar larvae of *Spodoptera exigua* H. (Boisduval). Compound **1** was active at 1000 ppm, corresponding to a concentration of 20 μg/cm^2, which was weak compared to those of the meliacarpinins (50 ppm) and azedarachins (200-400 ppm),[9] isolated from the same plant.

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References and Notes

1 D. E. Champagne, O. Koul, M. B. Isman, G. G. E. Scudder, and G. H. N. Towers, *Phytochemistry*, **31**, 377 (1992).

2 M. Nakatani, J. C. James, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 1228 (1981); M. Nakatani, M. Okamoto, and T. Hase, *Heterocycles*, **22**, 2335 (1984).

3 R. C. Huang, H. Okamura, T. Iwagawa, and M. Nakatani, *Bull. Chem. Soc. Jpn.*, **67**, 2468 (1994); M. Nakatani, R. C. Huang, H. Okamura, T. Iwagawa, K. Tadera, and H. Naoki, *Tetrahedron*, **51**, 11731 (1995).

4 M. Nakatani, J. -B. Zhou, T. Iwagawa, and H. Okamura, *Heterocycles*, **38**, 2407 (1994); J. -B. Zhou, H. Okamura, T. Iwagawa, Y. Nakamura, N. Nakayama, K. Tadera, and M. Nakatani, *Heterocycles*, **41**, 2795 (1995); M. Nakatani, J. -B. Zhou, N. Nakayama, H. Okamura, and T. Iwagawa, *Phytochemistry*, **41**, 739 (1996); J. -B. Zhou, Y. Minami, F. Yagi, T. Tadera, and M. Nakatani, *Phytochemistry*, **46**, 911 (1997); J. -B. Zhou, Y. Minami, F. Yagi, K. Tadera, and M. Nakatani, *Heterocycles*, **45**, 1781 (1997); J. -B. Zhou, K. Tadera, Y. Minami, F. Yagi, J. Kurawaki, K. Takezaki, and M. Nakatani, *Biosci. Biotech. Biochem.*, **62**, 496 (1998).

5 An amorphous powder; UV λ_{max} nm (ε): 219 (9000); CD: Δε -5.7 (215 nm).

6 J. N. Bilton, H. B. Broughton, S. V. Ley, Z. Lidert, E. D. Morgan, H. S. Rzepa, and R. N. Sheppard, *J. Chem. Soc., Chem. Commun.*, **1985**, 968.

7 K. Mitsunaga, K. Koike, K. Ishii, T. Ohmoto, A. Uchida, I. Oonishi, and Y. Ohashi, *Tetrahedron Lett.*, **34**, 6415 (1993).

8 K. Wada and K. Munakata, K. *J. Agr. Food Chem.*, **16**, 471 (1968).

9 R. C. Huang, J-B. Zhou, H. Suenaga, K. Takezaki, K. Tadera, and M. Nakatani, *Biosci. Biotech. Biochem.*, **59**, 1755 (1995).