PERGAMON

Phytochemistry 52 (1999) 1117–1119

PHYTOCHEMISTRY

Isolation of a new tetranortriterpenoid from the uncrushed green leaves of *Azadirachta indica*

T.R. Govindachari[a,*], R. Malathi[b], Geetha Gopalakrishnan[a], G. Suresh[a], S.S. Rajan[b]

[a]*Centre for Agrochemical Research, SPIC Science Foundation, 111 Mount Road, Madras, 600 032, India*
[b]*Department of Crystallography and Biophysics, University of Madras, Madras, 600 025, India*

Received 11 February 1999; accepted 9 June 1999

Abstract

14,15-Epoxynimonol, a new tetranortriterpenoid, was obtained from the fresh green whole leaves of *Azadirachta indica* and its structure has been assigned on the basis of spectral data. The 14,15-epoxide was assigned β-configuration by comparison with cedrelone. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Azadirachta indica; Nimonol; 14; 15-β-Epoxynimonol

1. Introduction

Various parts of *Azadirachta indica* A. Juss. (Meliaceae) were used in traditional medicine (Ketkar & Ketkar, 1995) and extensive research has resulted in the isolation of tetranortriterpenoids (Kraus, 1995). A number of them have been shown to have insect antifeedant, insect growth regulatory, antifungal, antimalarial and antiviral properties (Eppler, 1995; Locke, 1995; Schmutterer, 1995). In continuation of the work on the isolation of triterpenoids from the uncrushed green leaves, we have isolated a new tetranortriterpenoid (**1**), the structure elucidation of which is presented in this paper.

2. Results and discussion

In the present investigation, we have isolated a new tetranortriterpenoid, 14-15-β-epoxynimonol along with nimonol (Suresh, Narasimhan & Palani, 1997), isomeldenin (Pachapurkar, Kornule & Narayanan, 1974), 3-desacetylnimbin, nimbolide (Ekong, 1967) and homoazadiradione (Bruhn, Bokel & Kraus, 1984) from the uncrushed green leaves by preparative HPLC. 3-Desacetylnimbin is reported from the leaves for the first time.

14,15-β-Epoxynimonol (**1**) has a molecular formula $C_{28}H_{36}O_6$ (FAB-HR MS) (M + 1) 469.261660. The ^1H NMR and ^{13}C NMR spectral details of **1** are presented in Tables 1 and 2. The proton connectivities were established by consultation with ^1H–^1H COSY NMR data. The nature of the carbons was established by DEPT experiments. The proton, carbon connectivites were established with ^1H–^{13}C Hetero COSY NMR data.

Compound **1** differed from nimonol (Schmutterer, 1995) by having one more oxygen atom. ^1H spectra of compound **1**, revealed the chemical shift of H-15 appearing as a singlet at 3.49 ppm. In nimonol, H-15 was an olefinic proton appeared as a doublet of a doublet at 5.42 ppm. The ^{13}C NMR spectrum of **1** revealed the presence of an epoxide (C-14, 73.03 ppm and C-15, 57.39 ppm), while nimonol showed the presence of an olefinic bond between C-14 and C-15 (C-14, 158.53 ppm and C-15, 119.55 ppm). In cedrelone, a tetranortriterpenoid from *Cedrela toona* (= *Toona ciliata*), whose structure has also been determined by X-ray crystallography (Grant, Hamilton, Hamor, Robertson & Sim, 1963), the epoxide functionality at C-14–C-15 has been shown to be β oriented and the

---

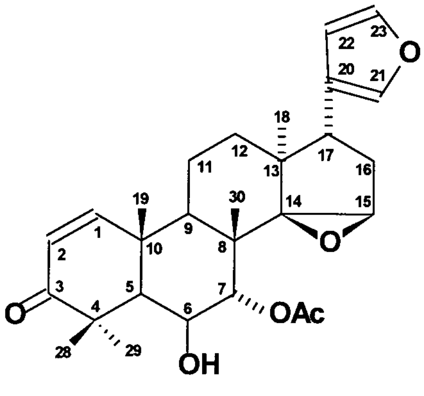
* Corresponding author.

0031-9422/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(99)00367-2

Table 1
^{1}H NMR data spectral data for nimonol and compound **1** (200 MHz, δ values in $CDCl_3$

Proton	Compound **1**	Nimonol
H-1	7.12 (1H, *d*, 9.9)	7.12 (1H, *d*, 10.06)
H-2	5.92 (1H, *d*, 9.9)	5.90 (1H, *d*, 10.06)
H-5	2.22 (1H, *d*, 11.69)	2.21 (1H, *d*, 11.65)
H-6	4.32 (1H, *dd*, 11.61, 3.04)	4.38 (1H, *dd*, 11.65, 2.37)
H-7	4.93 (1H, *d*, 2.63)	5.36 (1H, *d*, 2.37)
H-9	2.62 (1H, *m*)	2.21 (1H, *m*)
H-11	1.86 (2H, *m*)	1.82 (2H, *m*)
H-12	1.86 (2H, *m*)	1.82 (2H, *m*)
H-15a	3.49 (1H, *s*)	5.42 (1H, *dd*, 1.82, 2.84)
H-16a	1.60 (1H, *m*)	1.7 (1H, *m*)
H-16b	2.13 (1H, *m*)	2.4 (1H, *m*)
H-17	2.62 (1H, *m*)	2.83 (1H, *m*)
H-21	7.38(1H, *t*, 1.6)	7.26 (1H, *m*)
H-22	6.17 (1H, *br s*)	6.29 (1H, *m*)
H-23	7.12 (1H, *br s*)	7.39 (1H, *m*)
6-OH	1.69 (*br s*)	
OAc	2.15 (3H, *s*)	2.05 (3H, *s*)
C-methyls	0.97 (3H, *s*)	0.82 (3H, *s*)
	1.13 (3H, *s*)	1.14 (3H, *s*)
	1.17 (3H, *s*)	1.27 (3H, *s*)
	1.31 (3H, *s*)	1.31 (3H, *s*)
	1.38 (3H, *s*)	1.41 (3H, *s*)

H-15 proton does not show any coupling with the neighbouring methylene group (C-16). In the NMR spectrum of compound **1**, a singlet is observed for H-15 at 3.49 ppm and does not show any coupling with H-16 protons (^{1}H–^{1}H COSY). Accordingly, the compound **1** has been assigned the structure with β configuration of epoxide as in cedrelone.



1

3. Experimental

Fresh, uncrushed, green neem leaves (5 kg) were dipped in hexane (25 l) for 24 h and the decanted *n*-hexane extract was concentrated to 1 l in vacuo. The hexane extract was partitioned with 95% MeOH. The MeOH extract was concentrated to dryness in vacuo leaving a residue (16 g), which was used for the isolation of compound **1**.

3.1. Isolation of 14,15-β-epoxynimonol 1

1.3 g of this residue was dissolved in MeOH (4 ml), filtered through a Millipore filter (0.25 μm), and subjected to prep. HPLC (Shimadzu ODS column, 20 mm × 25 cm, 215 nm) using $MeOH:H_2O$ (70:30) as eluent at a flow rate of 15 ml min^{-1}. Twelve such runs were performed. Eight peaks with retention times 6.9, 11.9, 13.7, 17.8, 22.3, 31.4, 46.6 and 60.4 min, respectively, were collected and concentrated. The peaks eluting at 6.9, 17.8 and 31.4 min. on analytical HPLC (Shimadzu C_{18} RP 4.6 mm × 25 cm, 215 nm) using acetonitrile–water (50:50) at a flow rate of 1 ml min^{-1} were shown to be a complex mixture which was not further investigated. The peaks eluting at 11.9 min (255 mg) and 13.7 min (30 mg) were identified to be a mixture of desacetylnimbin and nimbolide

Table 2
^{13}C NMR spectral data for nimonol and compound **1** (50 MHz, δ values in $CDCl_3$)

^{13}C	Compound **1**	Nimonol
C-1	157.60	157.40
C-2	126.26	126.14
C-3	205.84	205.96
C-4	40.28	40.51
C-5	50.41	49.88
C-6	68.73	68.08
C-7	77.74	79.08
C-8	42.92	45.43
C-9	39.04	37.13
C-10	41.87	43.11
C-11	16.47	16.39
C-12	29.31	32.72
C-13	45.68	47.08
C-14	73.03	158.53
C-15	57.39	119.55
C-16	32.13	34.30
C-17	39.41	51.62
C-20	123.63	124.37
C-21	142.96	142.57
C-22	110.92	110.94
C-23	139.56	139.64
OAc	171.74	172.02
OAc	21.40	21.16
C-Methyls	21.86	27.08
	21.65	20.84
	19.31	20.23
	31.90	31.87
	19.97	20.79

which were separated by pooling them together and subjecting to another preparative HPLC. The peak eluting at 46.6 min and 60.4 min were identified to be nimonol (2.87 g) and isomeldenin (720 mg), respectively. The peak eluting at 22.3 min on evaporation yielded a solid (51 mg). This was subjected to preparative HPLC on an RP18 Column (E-Merck, ODS, 10 μm, 9.6 mm × 25 cm) using acetonitrile–water (40:60) as an eluent at a flow rate of 10 ml min^{-1}. Two peaks with retention times 52.9 and 74.8 min eluted which were collected and concentrated. The peak eluting at 52.9 min (8 mg) was identified to be 4α, 6α-dihydroxy-A-homo-azadiradione. The peak eluting at 74.8 min was collected and concentrated in vacuo to yield **1** (13 mg) as a solid. m.p.: 180–82°C; $[\alpha]_D = +33°$ ($CHCl_3$, C = 0.5); IR: ν_{CHCl_3} cm^{-1} 3400, 3100, 2912, 2816, 1792, 1718, 1660, 1593, 1449, 1372, 1254, 1084, 1062 and 646; ^1H NMR and ^{13}C NMR: See Tables 1 and 2; Mass: FAB Mass [M + 1] requires 469.259014 obs. 469.261660.

Acknowledgements

We thank the department of Biotechnology, Government of India for funding a project on antifungal compounds from Meliaceae and Simaroubaceae. SSR thanks the Department of Science and Technology for financial assistance.

References

Bruhn, A., Bockel, M., & Kraus, W. (1984). *Tetrahedron Letters, 25*, 3691.

Ekong, D. E. U. (1967). *Journal of Chemical Society Chemical Communications*, 808.

Eppler, A. (1995). In H. Schmutterer, *The neem tree: source of unique natural products for integrated pest management, medicine, industry and other purposes* (p. 93). Weinheim: VCH.

Grant, I. J., Hamilton, J. A., Hamor, T. A., Robertson, J. M., & Sim, G. A. (1963). *J. Chem. Soc.*, 2506.

Ketkar, A. Y., & Ketkar, C. M. (1995). In H. Schmutterer, *The neem tree: source of unique natural products for integrated pest management, medicine, industry and other purposes* (p. 518). Weinheim: VCH.

Kraus, W. (1995). In H. Schmutterer, *The neem tree: source of unique natural products for integrated pest management, medicine, industry and other purposes* (p. 37). Weinheim: VCH.

Locke, J. C. (1995). In H. Schmutterer, *The neem tree: source of unique natural products integrated pest management, medicine, industry and other purposes* (p. 118). Weinheim: VCH.

Pachapurkar, R. V., Kornule, P. M., & Narayanan, C. R. (1974). *Chemistry Letters*, *4*, 357.

Schmutterer, H. (1995). *The neem tree: source of unique natural products for integrated pest management, medicine, industry and other purposes* (p. 127). Weinheim: VCH.

Suresh, G., Narasimhan, N. S., & Palani, N. (1997). *Phytochemistry*, *45*, 807.