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13,14-DESEPOXYAZADIRACHTIN-A, A TETRANORTRITERPENOID FROM *AZADIRACHTA INDICA*

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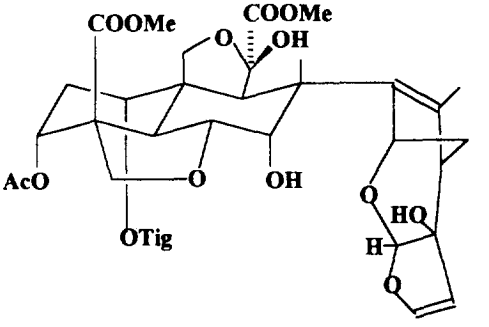
Key Word Index—*Azadirachta indica*; Meliaceae; desepoxyazadirachtin-A; azadirachtin-A; tetranortriterpenoid.

Abstract—A new tetranortriterpenoid, 13,14-desepoxyazadirachtin-A, has been isolated from neem kernel extract by preparative HPLC and its structure established by spectroscopic methods.

INTRODUCTION

In several publications from this laboratory [1–6] we have established the superiority of the preparative HPLC techniques in the isolation and purification of a number of triterpenoids present in neem kernel extract and in neem oil. Herein we report the isolation of yet another tetranortriterpenoid, whose structure was established as 13,14-desepoxyazadirachtin-A, by spectral methods.



1 13,14-Desepoxyazadirachtin-A

RESULTS AND DISCUSSION

In a HPLC run on the total triterpenoids of neem kernel or oil, a large number of peaks are seen. Some of them, on reanalysis using a solvent mixture of greater resolving power, give single peaks from which pure compounds can be isolated. Some other peaks resolve into a large number of peaks and identifying them all would require a major effort. In the HPLC analysis of neem kernel extract we encountered two peaks eluting after azadirachtin-B. One peak (r_t 29.8 min) proved to be complex in analytical HPLC and was not examined further. The other peak (r_t 33.2 min) contained reasonably pure material as shown by analytical HPLC. This was further submitted to another preparative HPLC, using a semi-preparative column, and material from the major peak (r_t 25 min) in this chromatogram was collected, eschewing minor peaks. By this procedure we were able to isolate a pure compound 13,14-desepoxyazadirachtin-A (**1**) as a solid, mp 187°, which is the subject of study in this paper.

This compound (**1**) on FAB HRMS analysis had a molecular formula $C_{35}H_{44}O_{15}$ which differed from azadirachtin-A only by having one oxygen atom less. In Tables 1 and 2 the NMR data on this new compound have been presented along with that of azadirachtin-A. Both in the proton and ^{13}C spectra, there were hardly any differences in the chemical shifts except that the methyl groups at C-13(C-18) and C-8 (C-30) appeared as singlets at 1.45 and 1.57 ppm compared to the same methyl groups in azadirachtin-A at 2.01 (*s*) and 1.74 (*s*). When the epoxy oxygen bonding C-13-C-14 in azadirachtin-A is absent there is upfield shift of both these groups. In the C-13 spectrum, both compounds have almost identical carbon resonances, except that there are signals at 92.3 ppm and at 94.5 ppm, which are sp_2 carbons, present in the new compound (**1**) and absent in azadirachtin-A. Taken in conjunction with the FAB HRMS data, the new compound should be 13,14-desepoxyazadirachtin-A, another addition to the group of azadirachtins present in neem kernel extract [7].

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Table 1. ^{1}H NMR spectral data for compounds of Azadirachtin-A (400 MHz) and **1** (600 MHz) (δ values in $CDCl_3$)

Proton	Azadirachtin-A	Compound **1**
H-1	4.75(*dd*, 2.9, 3.1)	4.67 (*dd*, 2.9, 3.1)
H-2	2.34(*ddd*, 16.7, 2.9, 2.7)	2.2(*ddd*, 16.7, 2.9, 2.7)
H-2	2.13(*ddd*16.7, 3.1, 2.9)	2.12(16.7, 3.1, 2.7)
H-3	5.50(*dd*, 2.7, 2.9)	5.66(*dd*, 2.7, 2.9)
H-5	3.35(*d*, 12.5)	3.14(*d*, 12.5)
H-6	4.60(*dd*, 12.5, 2.7)	4.37(*dd*, 12.6, 2.8)
H-7	4.75(*d*, 2.7)	4.56(*d*, 2.8)
H-9	3.34(*s*)	3.32(*s*)
H-11	—	—
H-15	4.67(*d*, 3.4)	4.68(*br s*)
H-16α	1.73(*ddd*, 13.0, 3.4, 5.1)	1.73(*ddd*, 13.3, 3.4, 5.1)
H-16β	1.31(*d*, 13.0)	1.29(*d*, 13.3)
H-17	2.38(*d*, 5.1)	2.55(*d*, 6.9)
H-18	2.01(*s*)	1.45(*s*)
H-19α	3.63(*d*, 9.6)	3.54(*d*, 9.6)
H-19β	4.15(*d*, 9.6)	3.99(*d*, 9.6)
H-21	5.65(*s*)	5.64(*s*)
H-22	5.05(*d*, 2.9)	4.89(*d*, 2.9)
H-23	6.46(*d*, 2.9)	6.39(*d*, 2.9)
H-28	4.08(*d*, 9.0)	3.98(*d*, 8.9)
H-28	3.76(*d*, 9.0)	3.58(*d*, 8.9)
H-29	—	—
H-30	1.74(*s*)	1.57(*s*)
12-OMe	3.68(*s*)	3.81(*s*)
29-OMe	3.76(*s*)	3.76(*s*)
OAc	1.95(*s*)	1.85(*s*)
Tigloyl		
H-3′	6.93(*qq*, 7.0, 1.5)	6.91(*qq*, 6.9, 1.4)
H-4′	1.78(*dq*, 7.0, 1.1)	1.76(*dq*, 7.0, 1.1)
H-5′	1.85(*dq*, 1.5, 1.1)	1.88(*dq*, 1.5, 1.1)

The three hydroxyl groups (7-OH, 11-OH and 20-OH) appeared at 2.58, 2.52 and 3.72 ppm which are exchangeable with D_2O. According to Rembold *et al.* [8, 9] desepoxyazadirachtin-B (called azadirachtin-G) is present in neem kernel extract, but neither details of its isolation nor structural characterisation have been published.

EXPERIMENTAL

Neem kernel extract enriched to 25% azadirachtin content as described earlier [2] was employed for the isolation of the new compound.

Isolation of 13,14-*Desepoxyazadirachtin-A* **1**. Five hundred mg of this extract 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 H_2O–MeOH–acetonitrile (10:7:3) as eluent at a flow rate of 10 ml min^{-1}. Four different peaks were collected with retention times 17.7, 23.9, 29.8 and 33.9 min, respectively, after which the column was washed with MeOH to elute the less polar compounds like azadiradione, nimbin, salannin, etc. from the column whose isolation from the neem kernel extract have been reported earlier [4]. The peak eluting at 17.7 min was identified to be a mixture of azadirachtins A and D (102 mg) and at 23.9 min to be azadirachtin B (25 mg). The peak eluting at 29.8 min on analytical HPLC (Shimadzu C_{18} RP 4.6 mm × 25 cm, 215 nm) using acetonitrile–H_2O (7:13) at a flow rate of 1 ml min^{-1} proved to be a complex mixture which was not further investigated. The peak eluting at 33.9 min on evaporation yielded a solid (56.2 mg). This was subjected in 5 mg lots to a further HPLC on a semiprep. column (LKB Ultropac Column, TSK ODS-120T 10 μm, 7.8 mm × 30 cm) using acetonitrile–H_2O (7:13) as eluent at a flow rate of 3 ml min^{-1}. The major peak eluting at 25.08 min under these conditions was collected and concentrated *in vacuo* to yield **1** (23 mg) as a solid. mp 187°; $[\alpha]_D$ +13.64° ($CHCl_3$, C = 0.44); UV λ_{max} (EtOH) 214.2 nm ($\varepsilon = 6.272 \times 10^4$); IR ν^{Kbr} cm^{-1} 3410, 1718, 1676, 1599; ^{1}H NMR and ^{13}C NMR: See Tables 1 and 2; Mass: FAB Mass [M-1] requires 703.260196. obs. 703.263268.

Table 2. ^{13}C NMR spectral data for compounds Azadirachtin-A (100 MHz) and 1 (150 MHz) (δ values in $CDCl_3$)

Carbon	Azadirachtin-A	Compound **1**
C-1	70.51	70.52
C-2	29.75	29.91
C-3	66.96	68.23
C-4	52.49	51.34
C-5	36.99	34.22
C-6	73.86	72.56
C-7	74.31	72.92
C-8	45.45	47.91
C-9	44.66	47.56
C-10	50.18	51.33
C-11	104.19	109.12
C-12	171.83	172.73
C-13	68.62	92.36
C-14	69.96	94.92
C-15	76.45	76.83
C-16	25.02	25.33
C-17	48.66	48.57
C-18	18.44	18.48
C-19	69.05	69.34
C-20	83.58	86.36
C-21	108.67	109.12
C-22	107.44	107.71
C-23	146.99	145.95
C-28	71.15	70.52
C-29	173.20	175.66
C-30	21.33	20.95
COOMe	53.28	53.23
COOMe	52.78	52.64
OAc	169.78	166.19
OAc	20.89	20.92
Tigloyl		
C-1′	166.22	163.26
C-2′	128.60	127.71
C-3′	137.50	139.58
C-4′	14.29	14.72
C-5′	11.94	12.15

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REFERENCES

1. Govindachari, T. R., Sandhya, G. and Ganesh Raj, S. P., *Journal of Chromatography*, 1990, **513**, 389.
2. Govindachari, T. R., Sandhya, G. and Ganesh Raj, S. P., *Chromatographia*, 1991, **31**, 303.
3. Govindachari, T. R., Sandhya, G. and Ganesh Raj, S. P., *Journal of Natural Products*, 1992, **55**, 596.
4. Govindachari, T. R., Sandhya, G. and Ganesh Raj, S. P., *Indian Journal of Chemistry*, 1992, **31B**, 295.
5. Govindachari, T. R., Suresh, G. and Geetha Gopalakrishnan., *Journal of Liquid Chromatography*, 1995, **18**, 3465.
6. Govindachari, T. R., Geetha Gopalakrishnan. and Suresh, G., *Journal of Liquid Chromatography and Related Technology*, 1996, **19**, 1729.
7. Kraus, W., in *The Neem Tree*, ed. H. Schmutterer. VCH, Weinheim, 1995, p. 35.
8. Rembold, H., in *1988 Focus on Phytochemical Pesticides, Vol. 1, The Neem Tree*, ed. M. Jacobson. CRC Press, FL, 1989, p. 47.
9. Rembold, H., in *Insecticides of Plant Origin*, ed. J. T. Arnason, B. J. R. Philogene and P. Morand. ACS Symposium Series 387, American Chemical Society, Washington, DC, 19XX, p. 150.