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NIMBOCINOL AND 17-EPINIMBOCINOL FROM THE NIMBIDIN FRACTION OF NEEM OIL

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Key Word Index—*Azadirachta indica*; Meliaceae; methanol extract; nimbidin fraction, nimbocinol and 17-epinimbocinol.

Abstract—A new tetranortriterpene alcohol has been isolated from nimbidin fraction of the methanol extract of neem oil. Its structure was established by spectral studies and conversion to 17-epiazadiradione by acetylation.

Azadirachta indica Juss (syn *Melia azadirachta* L; *Melia indica margosa*) known as 'neem' or 'nimba' is widely distributed in Asia, Africa and other tropical areas [1–5]. The biological properties associated with various parts of the plant have led to several investigations of its constituents. Some of the constituents of neem have recently been shown to possess significant pesticidal activity [6, 7]. Although the nimbidin fraction of the oil was found to exhibit anti-arthritic, anti-inflammatory [8] and anti-ulcer properties [9], there is no report on the structure of its constituents. We report herein the isolation and identification of a new tetranortriterpene alcohol, 17-epinimbocinol (**1**) and a known [10] alcohol nimbocinol (**2**) from the nimbidin fraction [14] of the neem oil.

RESULTS AND DISCUSSION

17-Epinimbocinol (**1**) has molecular formula, $C_{26}H_{32}O_4$ (mass spectral and elemental analysis). UV spectrum showed maxima at λ_{max} 237 and 222 (sh) nm; IR spectrum showed bands at ν_{max} 3515 (OH), 1680 and 1670 (α,β-unsaturated ketone), 3140, 1590 and 870 cm^{-1} (furan ring); mass spectrum showed peaks at m/z 408 $[M]^+$, 393 $[M-15]^+$, 390 $[M-18]^+$, 375 $[M-18-15]^+$, and 137 (ring A with α,β-unsaturated ketone).

The ^1H NMR spectrum of compound **1** (Table 1) showed two one proton AB doublets at δ7.13 and 5.86 ($J=10.2$ Hz) attributed to H_1 and H_2 of 1-en-3-one system of ring A; singlet at δ6.03 due to H_{15} of 14-en-16-one system of ring D; three one proton multiplets at δ6.07 (H_{22}), 7.35 (H_{21}), 7.20 (H_{23}); a one proton singlet at δ3.36 (H_{17}) and a three proton singlet at 1.47 (C_{13}Me). Thus the data obtained for compound **1** showed a close structural resemblance with nimbocinol. However, in nimbocinol H_{17} and C_{13}-Me appears at δ3.45 (*s*) and 1.03 (*s*) respectively. This led us to the structure of compound **1** as 17-epinimbocinol, the deshielding of C_{13}-Me is due to the β-oriented furan ring. Moreover the ^{13}C NMR spectral data (Table 2) are in agreement with the assigned structure. It may be noted in this connection that isolation of several 7α-hydroxy tetranortriterpenoids, have previously been reported from different species from the Meliaceae and Rutaceae [11, 12]. Acetylation of 17-epinimbocinol with acetic anhydride in the presence of *p*-toluene sulphonic acid afforded an acetate which was identical in every respect (mp, mmp, TLC, UV, IR, NMR and mass spectra) with 17-epiazadiradione [13]. Compound **2** was identified as nimbocinol (mp, TLC, UV, IR, ^1H NMR, ^{13}C NMR and mass spectra).

EXPERIMENTAL

Mps uncorr. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on 300 and 500 MHz instruments, respectively. The HPLC analysis was carried out using reverse phase C-18 col-

Table 1. ^1H NMR spectral data of compounds **1** and **2** (measured in $CDCl_3$ at 300 MHz, TMS as int. standard, chemical shifts in δ)

H	1	2
1	7.13 *d* (10.2, 1H)	7.13 *d* (10.2, 1H)
2	5.86 *d* (10.2, 1H)	5.87 *d* (10.2, 1H)
5	2.47 *dd* (3.12, 1H)	2.47 *dd* (3.12, 1H)
7	4.20 *m*	4.2 *m*
	1H ($W_{1/2}=7.5$ Hz)	1H ($W_{1/2}=7.5$ Hz)
9	2.53 *dd* (9.12, 1H)	2.56 *m* (1H)
15	6.03 *s* (1H)	6.07 *s* (1H)
17	3.36 *s* (1H)	3.45 *s* (1H)
21	7.35 *m* (1H)	7.47 *s* (1H)
22	6.07 *m* (1H)	6.27 *m* (1H)
23	7.20 *m* (1H)	7.43 *m* (1H)
Me	1.47 *s* (3H)	1.03 *s* (3H)
	1.24 *s* (3H)	1.23 *s* (3H)
	1.11 *s* (3H)	1.12 *s* (3H)
	1.17 *s* (3H)	1.17 *s* (3H)
	1.18 *s* (3H)	1.29 *s* (3H)

Coupling constant (J in Hz) in parentheses.

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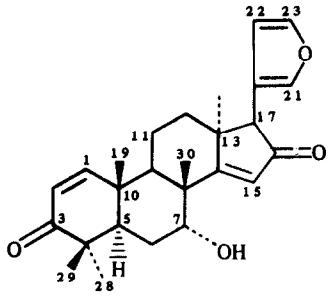

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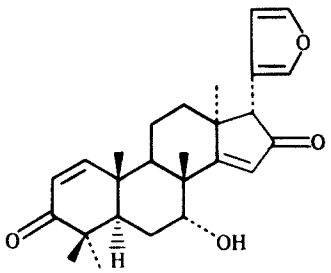

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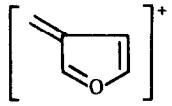
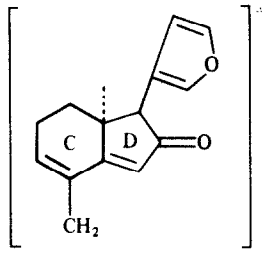


ion **a**



ion **b**

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (measured in $CDCl_3$ at 125 MHz, chemical shifts in δ)

C	1	2
1	157.28 *d*	157.18 *d*
2	125.65 *d*	125.63 *d*
3	204.58 *s*	204.59 *s*
4	46.77 *s*	44.10 *s*
5	36.31 *d*	36.46 *d*
6	27.01 *t*	25.47 *t*
7	71.75 *d*	71.53 *d*
8	47.79 *s*	44.43 *s*
9	44.53 *d*	44.43 *d*
10	44.16 *s*	40.08 *s*
11	15.53 *t*	15.63 *t*
12	26.27 *t*	30.41 *t*
13	40.14 *s*	48.09 *s*
14	197.07 *s*	194.05 *s*
15	123.70 *d*	123.36 *d*
16	207.91 *s*	205.38 *s*
17	59.75 *d*	60.70 *d*
18	31.34 *q*	26.50 *q*
19	19.02 *q*	18.95 *q*
20	121.33 *s*	118.42 *s*
21	142.98 *d*	142.59 *d*
22	110.06 *d*	111.08 *d*
23	140.26 *d*	141.49 *d*
28	21.39 *q*	21.35 *q*
29	25.68 *q*	25.87 *q*
30	26.26 *q*	26.96 *q*

umn, eluted isocratically with MeOH–MeCN–H_2O (8:1:1) with a flow rate of 0.2 ml min^{-1} and detection at 210 nm.

Isolation of nimbidin fraction from neem oil. The MeOH extract of industrial grade neem oil (400 g) was hydrolysed in a Parr-pressure reactor by adding equal amount of H_2O. After removing the fatty acids by vacuum dist. the residue obtained was further fractionated by solvent separation as described in ref. [14] to obtain the nimbidin equivalent fr. (5.56) which was then purified by CC over silica gel with C_6H_6–EtOAc (4:1) (0.47 g) and 7:3 (0.352 g) to give compounds, **2** and **1** respectively. On HPLC of nimbidin equivalent fraction and purified compounds, **1** and **2**, were eluted after 14.88 and 15.30 min respectively.

Compound **1** was crystallized from MeOH as needles mp 253–255° $[\alpha]_D^{30}$ −72° ($CHCl_3$; *c* 0.5); IR ν_{max}^{KBr} cm^{-1}, 3515 (OH), 1680 and 1670 (α,β-unsaturated carbonyl), 3140, 1590 and 870 cm^{-1} (furan ring).

^1H NMR (300 MHz, $CDCl_3$) (Table 1), ^{13}C NMR (125 MHz, $CDCl_3$) (Table 2); MS: *m/z* 408 $[M]^+$, Anal calcd for $C_{26}H_{32}O_4$; C-76.44, H-7.89%; found C-76.86, H-8.29%.

Acetylation of 17-epinimbocinol (**1**). A mixt. of 17-epinimbocinol (0.05 g) (**1**), Ac_2O (1.5 ml) and *p*-toluene sulphonic acid (0.05 g) was stirred at room temp. for 1 hr, after usual work-up furnished the acetyl derivative. It was identical with the sample of 17-epiazadiradione (mp, mmp and comparison of spectral data UV, IR ^1H NMR and mass).

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A DIHYDROXYKETOSTEROID FROM THE MARINE RED ALGA *HYPNEA MUSCIFORMIS*

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Key Word Index—*Hypnea musciformis*; Rhodophyta; red alga; dihydroxyketosteroid; 23-methyl steroid; 23-methyl-5β-cholesta-1,22-diene-11,20-dihydroxy-7-one.

Abstract—The isolation of a new dihydroxy ketosteroid is reported from the hexane extract of the marine red alga *Hypnea musciformis*. This compound has been characterized as 23-methyl-5β-cholesta-1,22-diene-11,20-dihydroxy-7-one based on spectral data and by comparison with its congeners.

INTRODUCTION

There has been continuing interest in the sterols and steroids of marine organisms ever since the earliest studies of Henze [1] and Doree [2] showing the potential for new sterols other than cholesterol. The discovery of many new marine sterols confirms the predictions made long ago by Bergmann [3] regarding their diversity. In the sterol composition of the red algae, C_{27} compounds were found to be major constituents in which cholesterol predominates. The presence of trace amounts of C_{26}, C_{27} and C_{29} compounds has been reported [4–6] and demosterol [7], a 3-keto steroid [8, 9] and a 3,6-diketo steroid [10] were documented in some species. 22-Dehydrocholesterol is reported to be present in relatively large amounts only in *Hypnea japonica* [11] and *Hypnea musciformis* [12]. We now report the isolation from *Hypnea musciformis* of 23-methyl-5β-cholesta-1,22-diene-11,20-dihydroxy-7-one (**1**). 5β-Cholest-3-en-7,11-dione (**3**) [13] and 5β-cholest-1-en-20-hydroxy-7,11-dione (**2**) [14] were previously reported from *Hypnea musciformis*.

RESULTS AND DISCUSSION

The cold hexane extract of the seaweed was chromatographed over silica gel by gradient elution (ethyl acetate–hexane). A crystalline compound (**1**) was obtained by elution with 30% ethyl acetate in hexane.

The steroidal nature of the compound **1** was revealed by the ^{1}H NMR spectrum and the mass spectral fragmentation. The ^{1}H NMR spectrum displayed signals at