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Two picrotoxin derivatives from *Anamirta cocculus*

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Abstract

The berries of the plant *Anamirta cocculus* afforded picrotin, picrotoxinin, methyl picrotoxate and two new sesquiterpene γ-lactones, dihydroxypicrotoxinin and picrotoxic acid, whose structures were determined by spectroscopic methods.

Keywords: Anamirta cocculus; Menispermaceae; Sesquiterpene lactones; Dihydroxypicrotoxinin; Picrotoxic acid; Picrotoxinin; Methyl picrotoxate; Picrotin

1. Introduction

Anamirta cocculus (Linn.) Wight et Arn., syn. *Anamirta paniculata* and commonly known as Jahre-mahi is a liana occurring in several regions of South–east Asia (Bentley & Trimen, 1980). In India, it is mainly found in Orissa, Eastern Bengal, Khasia hills, Deccan (Cuddapah, Malabar & Mysore), etc. The berries have been used in India to stupefy fish and to poison crows and very small quantities are sufficient for this purpose (Wealth of India, 1948; Drury, 1973). They have also been employed as cattle poison (Hooper & Field, 1937; Venkatachalan, 1945). The use of berries in small quantities is described in a number of pharmacopoeias (Osol, Pratt, & Altschule, 1960; Morton, 1977), though these are highly toxic and fatal if consumed by humans. The symptoms produced are colic pains, nausea, vomiting, tetanic convulsions and occasionally delirium (Wealth of India, 1948). The active poisonous principle of the berries is picrotoxin, a sesquiterpene mixture (Hegnauer, 1969; Taylor & Battersby, 1969; Boullay, 1980). Other reported compounds belonging to this class are picrotoxinin (Corey & Pearce, 1978), picrotin (Corey & Pearce, 1980) and methyl picrotoxate (Porter, 1967; Sarma, Rambabu, Anjaneyulu, & Rao, 1987; Pradhan, Mamdapur, & Sipahimalani, 1990). The present paper describes the isolation and structure of two new minor sesquiterpene lactones of the picrotoxane group from *A. cocculus* seeds, in addition to those previously reported.

2. Results and discussion

Three known sesquiterpene lactones, picrotoxinin (**1**), methyl picrotoxate (**2**) and picrotin (**3**), as well as two new sesquiterpene lactones, dihydroxypicrotoxinin (**4**) and picrotoxic acid (**5**), were isolated and characterised from the seeds of *A. cocculus* compounds **1** and **3** were the major constituents.

Dihydroxypicrotoxinin (**4**) was assigned the molecular formula $C_{15}H_{18}O_8$ ($[M]^+ = m/z$ 326 and elemental analysis). Its IR spectrum showed the presence of two γ-lactone moieties at 1790 and 1760 cm^{-1} and primary and tertiary hydroxyl groups at 3340 (broad) cm^{-1}.

The ^1H NMR spectrum exhibited two methyl singlets at δ 1.36 (C9–CH_3) and 1.24 (C7–CH_3) and the doublets of a –CH_2OH group at δ 3.45 and 3.60. Other signals are listed in Table 1 and indicated that **4** belonged to the picrotoxane group with two hydroxyls at C-8 and C-10 instead of the double bond present in picrotoxinin (**1**).

The ^{13}C NMR spectrum of **4** showed the presence of 15 carbons (Table 2) two of which were lactone carbonyl carbons at δ 176.8 and 173.5 and seven were oxygen-bearing carbons. **4** formed an acetonide (**6**) when reacted with dry acetone and anhydrous copper sulphate indicating the vicinal nature of the two hydroxyls. Thus, the structure of **4** was established for dihydroxypicrotoxinin.

Picrotoxic acid (**5**) was assigned the molecular formula $C_{15}H_{18}O_7$ ($[M]^+ = m/z$ 310 and elemental analysis). The presence of a γ-lactone moiety, a tertiary hydroxyl and a free carbonyl group were revealed by IR bands at 1786, 3446 and 1716 cm^{-1} respectively. The ^{13}C NMR spectrum of **5** showed the presence of 15 carbons (Table 2) of which two were carbonyl carbons at δ 176.0 and 170.5.

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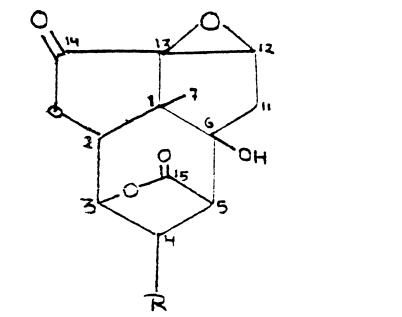

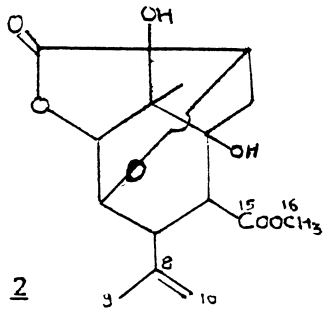

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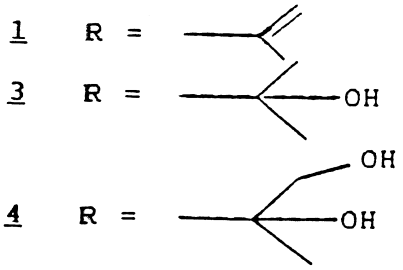
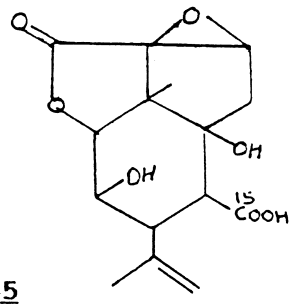


1 R =

3 R = OH

4 R = OH, OH



5

One of them was assigned to a γ-lactone and the other to a carboxyl group carbon.

In the ^{1}H NMR spectrum, singlets at δ 1.21 and 1.72 were attributed to the usual tertiary methyl on C-1 and the vinylic methyl on C-8, in addition two singlets at δ 4.88 and 4.85 represented the C-10 methylene and one oxymethine proton at δ 4.02 (Table 1).

Acetylation of **5** with Ac_2O and C_5H_5N yielded a monoacetate (**7**) as indicated by the appearance of new singlet at δ 2.00 and the downfield shift of the C-3 methine signal.

Picrotoxinin (**1**) was treated with dil. NaOH to give picrotoxic acid as described in an earlier paper (Hathway, 1957). Synthetically prepared **5** was found to be identical

Table 1
^{1}H NMR data of compounds **1**–**5** and **7**

H	1	2	3	4	5	7
2	4.94 d(3)	4.22 d(2)	–	4.50 d(3)	4.32 d(3)	4.90 d(3)
3	5.01 dd(5,3)	3.82 d(2)	–	5.06 dd(5,3)	4.02 dd(3,6)	4.60 dd(3,6)
4	3.42 bs ($W_{1/2}$=4)	2.98 d(12)	–	3.44 d(3)	3.40 bs ($W_{1/2}$=4)	3.45 m
5	2.93 d(5)	2.80 d(12)	4.84 d(4)	2.88 d(4)	2.61 d(12)	2.95 d(10)
6	1.60 s	1.43 s	1.54 s	1.89 s	1.48 s	1.49 s
7	1.23 s	1.21 s	1.20 s	1.24 s	1.21 s	1.23 s
9	1.90 s	1.76 s	1.40 s	1.36 s	1.72 s	1.71 s
10	4.99 s	4.87 s	1.40 s	3.45 d(12)	4.88 s	4.89 s
	4.77 s	4.83 s		3.60 d(12)	4.85 s	4.85 s
11	2.87 d(15)	3.09 d(15)	2.86 d(15)	2.99 d(12)	2.88 d(15)	2.95 d(15)
	2.02 dd(15,3)	2.19 dd(15,3)	2.12 dd(15,3)	2.07 dd(12,3)	2.08 dd(15,3)	2.15 dd(12,3)
12	3.70 d(3)	4.17 d(3)	3.75 d(3)	4.20 d(3)	3.68 d(3)	3.80 d(3)
16	–	3.69 s	–	–	–	–
17	–	–	–	–	–	2.00 s

Table 2
^{13}C NMR data of compounds **1**–**5**

C	1	2	3	4	5
1 s	66.20	58.20	69.50	70.50	60.00
2 d	81.40	81.85	79.20	82.80	83.00
3 d	82.50	79.70	82.20	79.60	81.50
4 d	45.10	47.20	52.40	51.90	47.50
5 d	63.70	47.80	63.10	63.40	45.00
6 s	74.50	85.50	74.50	74.90	87.00
7 q	10.80	10.35	10.50	10.40	11.50
8 s	141.80	141.70	86.10	86.30	145.00
9 q	23.20	22.10	28.50	23.90	22.50
10 t	112.20	112.80	29.90	70.10	114.00
11 t	42.90	42.90	44.00	44.50	43.50
12 d	52.50	72.80	53.80	49.80	52.00
13 s	50.10	80.30	52.70	50.70	48.50
14 s	176.70	176.10	176.30	176.80	176.00
15 s	175.20	173.30	175.10	173.50	170.50
16 q	–	52.30	–	–	–

Carbon multiplicity by DEPT for compounds **1** and **2**.

with naturally occurring picrotoxic acid by co-TLC, mmp, ^{1}H NMR, EIMS and IR. Thus picrotoxic acid was assigned as **5**.

2. Experimental

2.1. General

Mps: uncorr.; ^{1}H and ^{13}C NMR: Varian FT-80A (80 MHz), Bruker (200 MHz and 400 MHz), chemical shifts in δ (ppm) with TMS as int. standard. EIMS: 70 eV; CC: Silica gel (Ranbaxy, 60–120 mesh); TLC: Silica gel G (Ranbaxy); detection of spots by exposure to I_2 vapour and/or by spraying with 5% vanillin–sulphuric acid soln. followed by heating at 105° for 5 min.

2.2. Plant material

The seeds of the *A. cocculus* were purchased from local market and identified in our Botany Department, where a voucher specimen has been maintained.

2.2.1. Extraction and isolation

The air dried and powdered seeds (5.0 kg) were extracted with MeOH (5 × 10.0 l), in a perculator at room temp. After filtration, the combined dark brown soln. was evapd. to dryness under red. pres. below 60°. The methanolic extract was obtained as a dark brown gummy mass (280 g). A part of the MeOH extract (100 g) was chromatographed on a silica gel column and eluted successively with hexane, hexane–EtOAc (2:1), (1:1), (1:2), (1:3) mixtures and EtOAc. The eluates were monitored by TLC and grouped into 9 frs.

Fr 2 eluted with hexane–EtOAc (1:1) was rechromatographed on a silica gel column using hexane–EtOAc (3:1), (2:1), (1:1), (1:2) and (1:3) mixtures as eluate, yielding compound **1** (mp 210–12°, 300 mg), **2** (mp 174°, 60 mg) and **3** (mp 266–67°, 1.3 g), with identical mp, IR values, $[\alpha]_D$, and ^{1}H NMR to those of picrotoxinin, methyl picrotoxate and picrotin, respectively.

Fr 6 eluted with hexane–EtOAc (1:4) was rechromatographed on a silica gel column using hexane–EtOAc (1:1), (1:2), (1:3), EtOAc and EtOAc–MeOH (5%) mixtures as eluate yielding compounds **4** (40 mg) and **5** (25 mg).

2.2.2. Dihydroxypicrotoxinin (**4**)

Colourless needles, mp 260–62°; $[\alpha]_D^{17}$ −65° (c = 1.35 in EtOH); IR ν_{max}^{KBr} cm^{-1}: 3340 broad (–OH), 1790 and 1760 (for two γ-lactones), 1128; ^{1}H NMR (200 MHz, CD_3OD): Table 1; ^{13}C NMR (50 MHz, CD_3OD): Table 2; EIMS m/z (rel. int.): 326 $[M]^+$(10), 294(11), 276(6), 250(20), 232(32), 206(8), 185(14), 150(16), 135(45), 124(16), 110(38), 97(27), 83(14), 75(95), 67(12), 55(68), 43(100); (Found: C, 55.46; H, 5.86. $C_{15}H_{18}O_8$ requires: C, 55.21; H, 5.52%).

2.2.3. Acetonide (**6**) of **4**

4 (10 mg) in dry Me_2CO (1.5 ml) and anhydrous $CuSO_4$ (2 × 50 mg) were stirred together for 6 h. The reaction mixture was filtered and the filtrate was evaporated, again dissolved in $CHCl_3$ and filtered. The filtrate was concentrated and crystallised to give colourless needles (7 mg), mp 210–12°; ^{1}H NMR (80 MHz, $CDCl_3$): δ 1.26, 1.29 (2 × CH_3, acetonide) and other proton signals; EIMS m/z (rel. int.): 334 $[M]^+$ (25).

2.2.4. Picrotoxic acid (**5**)

White crystalline needles, mp 208–10°; IR ν_{max}^{KBr} cm^{-1}: 3446 (t-OH), 1786 (for one γ-lactone), 1716 (–COOH); ^{1}H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (20 MHz, DMSO-d_6): Table 2; EIMS m/z (rel. int.): 310 $[M]^+$ (12), 292(41), 273(24), 264(14), 247(16), 218(22), 186(27), 169(29), 152(49), 138(63), 125(52), 111(100), 95(68), 79(29), 67(59), 55(52), 44(65); (Found: C, 58.34; H, 5.76. $C_{15}H_{18}O_7$ requires: C, 58.06; H, 5.81%).

1 (10 mg) was treated with 0.1 N NaOH (5 ml) in MeOH (5 ml) at room temp for 25 h. After removal of excess NaOH with 0.01 N HCl, the reaction product was treated with Et_2O and the organic layer seperated and evaporated to yield picrotoxic acid (**5**, 6 mg), identical with that described above (by co-TLC, mmp, ^{1}H NMR, EIMS).

2.2.5. Mono-acetate (**7**) of **5**

Compound **5** (5 mg) was dissolved in Ac_2O (0.5 ml)-C_5H_5N (0.5 ml) and left to stand at room temperature. Usual workup yielded a colourless amorphous compound (**7**); IR ν_{max}^{KBr} cm^{-1}: 3406 (t-OH), 1796 (one γ-lactone), 1737 (ester C=O), 1719 (–COOH); ^{1}H NMR (80

MHz, $CDCl_3$): Table 1; EIMS m/z (rel. int.): 352 $[M]^+$ (11).

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References

Bentley, R., & Trimen, H. (1980). *Medicinal plants-I* (Chap. 14).
Boullay, P. F. G. (1980). *Ann. Chim. Phys. (Paris), 209,* 1811.
Corey, E. J., Pearce, H. L. (1978). *J. Am. Chem. Soc. 101,* 5841.
Corey, E. J., Pearce, H. L. (1980). *Tetrahedron Lett. 21,* 1823.
Drury, H. (1973). *The useful plants of India* (p. 512). London: William H. Allen & Co.
Hathway, D. E. (1957). *J. Chem. Soc.* 4953.
Hegnauer, R. (1969). *Chemotaxonomie der Pflanzen* (Vol. 5, p. 88). Basel, Stuttgart: Birkhaauser Verlay.
Hooper, D., Field, H. (1937). Useful plants and drugs of Iran and Iraq. *Bot. Ser, Field Mus. Nat. Hist. Chicago, 9,* 241 Pub. 387.
Morton, J. F. (1977). *Major medicinal plants* (p. 93). USA: Charles C. Thomas Publ.
Osol, A., Pratt, R., & Altschule, M. D. (1960). *The US Dispensatory and Physicians Pharmacology*, 1277.
Porter, I. A. (1967). *Chem. Rews., 67,* 441.
Pradhan, P., Mamdapur, V. R., Sipahimalani (1990). *Indian J. Chem., 29B,* 676.
Sarma, N. S., Rambabu, M., Anjaneyulu, A. S. R., Rao, C. B. S. (1987). *Indian J. Chem., 26B,* 189.
Taylor, W. I., & Battersby, A. R. (1969). In C. J. Coscia (Ed.), *Cyclo pentenoid terpene derivatives* (p. 147). New York: Marcel Dekker.
Venkatachalan (1945). *Mon. Bull. Banglore Cattle Soc., 4,* 47.
Wealth of India (1948). PID, CSIR (New Delhi), *1,* 75.