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Research Article

Isolation and Characterization of Compounds from Fruits of *Anamirta cocculus* (Linn.)

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ABSTRACT
Compounds from fruits of *Anamirta cocculus* were isolated and characterized. The phytoconstituents present in fruits were isolated by soxhlet extraction with methanol. The compounds were separated by column chromatography and characterized by chemical and spectroscopic methods. These compounds were identified as sesquiterpenoids and aliphatic amides containing carbonyl and hydroxyl functionalities namely,5,8-dihydroxy-12-methyl-2-oxo-6-(prop-1'-en-2'-yl)-3,11-dioxatetracyclo,dodecane-7carboxylicacid(CompoundA1),1-hydroxy-14 (2'-hydroxypropan-2'-yl)-13-methyl-4,7,10-trioxapentacyclo,tetradecane-6,11-dione (CompoundA2) & Methyl 1, 6 – dihydroxy-2-methyl-5-oxo-10-(prop-1'-en-2'-yl)-4,8-dioxatetracyclo,dodecane-11-carboxylate (CompoundA3) and two aliphatic amides namely (2Z,4Z)-N-methyltetracosa-2, 4-dienamide (Compound A4)& N –Ethyl-5-O -β-d –Glucopyranosyl pentanamide (CompoundA5) were isolated and characterized.

Keywords: *Anamirta cocculus*, Column chromatography, Spectroscopic methods, Sesquiterpene lactones, aliphatic amides.

INTRODUCTION

Anamirta cocculus is found in South East Asian and Indian Subcontinent and belongs to the family Menispermaceae[1].The seeds are known as *Cocculus indicus* (Fructuscocculi) and Indian berries. The plant possesses a strong, climbing shrub, with a corky, ash-colored bark having deep cracks or fissures. Fruit is a drupe, nearly spherical, 1cm in diameter when dry, smooth and hard[2]. It is distributed throughout India in dense forests and has been used in the indigenous system of medicine in curing different types of diseases like bronchitis, foul ulcers, dermatophytosis, phthisis, inflammation, fungal infections, vertigo, vitiated condition of vata and kapha[3], breast cancer[4], scabies[5] and antidote for morphine poisoning[6]. It has also been suggested to possess larvicidal[7] and antioxidant activity[8]. The fruit contains alkaloids menispermine and paramenispermine[6]. Four quaternary alkaloids berberine, palmatine, magnoflorine, columbamine and one tertiary alkaloid (-)-8-oxotetrahydropalmatine in an investigation of the non-quaternary alkaloid fraction of *Anamirta cocculus*[9]. One new triterpenoid, 2α, 3β, 23-trihydroxyolean-12-en-28-oate and 2α, 3β– dihydroxy-23- β-d-glucopyranosyloxyolean-12-en-28-oic acid, are reported from the stem of *Anamirta cocculus*[10].

MATERIALS AND METHODS

Experimental Section -General

Melting points were determined on labard scientific melting point apparatus. The IR spectra in KBr were recorded on Spectrum 400 Perkin Elmer. λ_{max} values were measured on UV-1800 Shimadzu spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Evance II400 MHz spectrophotometer and LC-MS on a Shimadzu spectrophotometer. The column chromatography was carried out on Silica gel G (60-120) for column chromatography activated at 110^{o} C for 1hr and TLC on silica gel G[11]. Spots were viewed in UV chamber and by iodine vapours. All the solvents and chemicals used were of Analytical reagent grade.

Plant Material

Fruits of *Anamirta cocculus* were collected from Wayanad district of Kerala in the month of December 2009, in a quantity sufficient for all the experiments. The plant material was authenticated by Dr. H. B. Singh, Scientist & Head, Raw Materials Herbarium & Museum, NISCAIR, Dr K. S. Krishnan marg, New Delhi and specimen was submitted and preserved for future reference [Voucher specimen No.1397/194]. The *Anamirta cocculus*fruits were washed under running tap water and shade dried for 15days. The shade dried fruits were powdered using a dry grinder to get the coarse powder (sieve no. 10/44). The powder was stored in air tight container for further use.

Extraction and isolation of the compounds

The fruits (4kg) shade dried, cleaned, powered, and defatted with light petroleum ether (28Ltr, b.p.60-80^{o}) by maceration for seven days. Filtered and filtrate was rejected and the residue was subjected to extraction in soxhlet apparatus with absolute methanol (40Ltr) for 36

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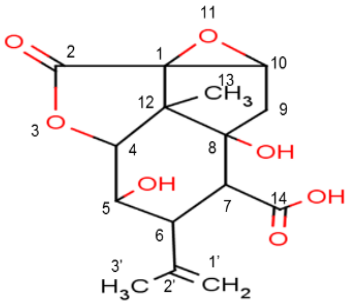
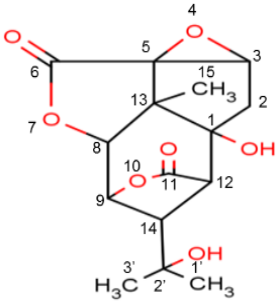
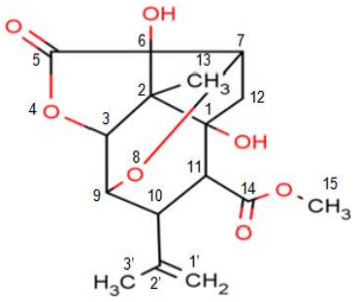


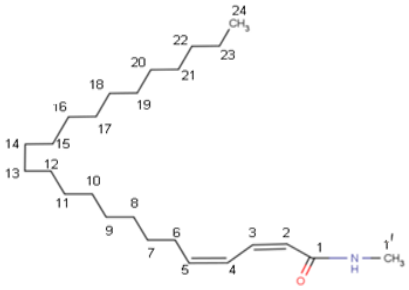
Compound A1



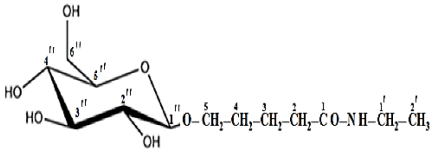
Compound A2



Compound A3



Compound A4



Compound A5

Figure 1: Compounds A1 to A5 isolated from methanolic extract of *Anamirtacocculus* (Linn.) fruits.

hrs. The methanolic extract was filtered and the combined dark green extract was concentrated to ¼ of its volume using a rotary vacuum evaporator to afford a thick dark green mass weighing 330 g and the percentage yield was calculated using the formula,% yield= weight of crude extract/initial weight of sample x100[12]. A part of the methanolic extract (100g) was chromatographed on a silica gel column and eluted by solvents of increasing polarity[13,14]. The elution was carried out successively with 100ml portions each of chloroform alone; chloroform: ethyl acetate graded mixtures (95:5,90:10,85:15…up to 100% ethyl acetate) ; ethyl acetate: methanol similar graded mixtures[15]. The fractions were combined based on their TLC pattern & individual compounds were further purified by preparative thin layer chromatography on silica gel G (for TLC)[16] in the presence of chloroform, ethyl acetate and methanol (9:1, 8:2, 7:3 and chloroform 100%) as mobile phase and recrystallized using methanol, yielding compound A1m.p. 208-210°C, 225mg; A2 m. p. 266-267°C, 326 mg; A3 m. p. 174°C, 280 mg; A4 m. p. 44-48°C, 340 mg & A5 m. p. 64-68°C, 410mg respectively. The pure compounds were subjected to characterization by UV, FTIR, ^{1}H NMR, ^{13}CNMR &LC-MS studies[17] and their structures are shown in Fig.1.

RESULTS

5,8-dihydroxy-12-methyl-2-oxo-6-(prop-1'-en-2'-yl)-3,11-dioxatetracyclo, dodecane-7carboxylic acid (CompoundA1)

M^{+}310, $C_{15}H_{18}O_7$ (225mg, chloroform: Ethyl acetate) furnished yellow coloured crystals, re-crystallized from methanol, m.p.208-210^{0}C, isolated from chloroform: Ethyl acetate (9:1v/v) fractions of the column.IR(K Br): 3431(t-OH), 2922(C-H), 2851(C-H), 1784.5 (for γ-lactone),1727.54 cm^{-1}(-COOH).^{1}H-NMR (400MH$_Z$ $CDCl_3$): δ 4.26 (1H,H-4),4.09(1H,H-5),3.5 (1H,H-6), 2.68 (1H,H-7), 2.89 and 2.09 (2H,H-9), 3.68 (1H,H-10),1.25(3H,H-13),4.91,4.87(2H,H-1'),1.79(3H,H-3'),3.12&3.46(2Hbrm,2xOH),7.72(1H,COOH).^{13}C-NMR(400MH$_Z$$CDCl_3$): C-1(47.0), C-2(175),C-4(82.3), C-5(80.31), C-6(48.0), C-7(42.0), C-8(85.8), C-9(42.46), C-10(52.3), C-12(58.62), C-13(10.67),C-14 (170.0), C-1' (112.83), C-2'(141.7), C-3' (22.66). LC–MS m/z(rel.int): 310+23(Na) Sodiated peak=333.UV$_{\lambda max}$: 222 nm (in chloroform).

1-hydroxy-14-(2'-hydroxypropan-2'-yl)-13-methyl-4,7,10-trioxapentacyclo, tetradecane- 6, 11-dione (Compound A2)

M^+310,$C_{15}H_{18}O_7$(326mg, Chloroform: Ethyl acetate) furnished yellow coloured crystals, re-crystallized from methanol, m. p. 266 - 267^0 C, isolated from chloroform : ethyl acetate (9:1v/v) fractions of the column.IR (KBr): 3448.26 (OH),2963(C-H), 2933 (C-H), 1787 (lactone stretch), 1731(-C=O), 1439 (C-H bend), 1305,1116, 991, 756.4cm^{-1}.^{1}H-NMR(400 MH_Z $CDCL_3$) : δ 2.76, 2.21 (2H,H-2), 3.72 (1H,H-3), 4.9 (1H,H-8), 3.85 (1H,H-9), 2.9 (1H,H-12), 3.44 (1H, H-14),1.33 (3H, H-15), 1.45 (3H, H-1'), 1.79 (3H, H-3'), 3.088 & 3.125 (2H, 2x OH).^{13}C-NMR(400 MH_Z $CDCL_3$): C1(72.8), C-2(44),C-3(52.4),C-5(50.8),C-6(174), C-8(79.8), C-9(85), C-11(169),C-12(61.57), C-13(71), C-14(50.2), C-15(10.47), C-1'(23.0) C-2'(86.1),C-3'(25.46). LC – MS m/z(rel.int): 310+23(Na) Sodiated peak = 333.UV λ_{max}: 223 nm (in chloroform).

Methyl 1,6-dihydroxy-2-methyl-5-oxo-10-(prop-1'-en-2'-yl)-4,8-dioxatetracyclo,1 dodecane-11-carboxylate(CompoundA3)

M^+324,$C_{16}H_{20}O_7$(280mg, Chloroform: Ethyl acetate) furnished dark brown coloured crystals, re-crystallized from methanol, m.p.174^{0}C, isolated from chloroform: ethyl acetate (9:1v/v) fractions of the column. IR (K Br):3378 (OH), 1782.6 (γ-lactone),1727(ester),1576.6(C=C),1115.6(C=C),1032(C-O),772cm^{-1} (C-O). ^{1}H-NMR(400MH_Z $CDCL_3$): δ 4.24 (1H, H-3), 4.169 (1H, H-7), 3.9 (1H, H-9), 3.0 (1H, H-10), 2.79 (1H, H-11), 3.06, 2.17 (2H,H-12),1.28(3H,H-13), 3.65 (3H,H-15), 4.90,4.87(2H,H-1'),1.78(3H,H-3'),3.58&3.72 (2H,2OH). ^{13}C-NMR (400MH_Z $CDCL_3$):C-1(85.6), C-2(58.57), C-3(82.1), C-5(176.76),C-6(80.33), C-7(72.9),C-9(77.0),C-10(47.1),C-11(47.9), C-12(47.1), C-13(10.7), C-14(173.65), C-15(52.24), C-1' (112), C-2' (141.8), C-3'(22.68). LC – MS m/z(rel.int): 324+23(Na) Sodiated peak = 347.UVλ_{max}: 219 nm (in chloroform).

(2Z, 4Z)-N-methyltetracosa-2, 4-dienamide(Compound A4)

M^+377,$C_{25}H_{47}ON$(340mg, Chloroform:Ethyl acetate) furnished brown coloured crystals, re-crystallized from methanol, m.p.44-48^{0}C, isolated from chloroform: ethyl acetate (9:1v/v) fractions of the column.IR(KBr):2926.36(CH_2),2854.44(CH_3), 1787(C=O) 1729 (C=C), 1556 (N-H), 1378 (CH_3 – bend), 1304.51 (- CH saturated), 1280 (-CH scissoring), 1166(N-CH_3vibration), 1033(rocking - CH of HC=C), 822.6 cm^{-1} (rocking of CH saturated). ^{1}H-NMR (400MH_Z $CDCL_3$): δ 5.06 (1H,H-2), 4.91(1H,H-3), 4.87(1H,H-4), 4.83 (1H,H-5), 3.43-1.423 (36H,H-6to23), 0.8928(3H,H-24), 3.73(3H,H-1′), 7.2686(1H,NH).^{13}C-NMR (400MH_Z $CDCL_3$): C-1(167.66), C-2(130.92),C-3(132),C-4(128),C-5(141.8), C-6 to C-23(48.07-19.13),C-24(14.09), C-1'(48.6). LC–MS m/z(rel.int): 377+23(Na) Sodiated peak = 400.UVλ_{max}: 376 nm (in chloroform).

N-Ethyl-5-O-*β*-d-Glucopyranosylpentanamide (CompoundA5)

M^+307, $C_{13}H_{25}O_7N$ (410mg, Ethylacetate: Methanol) furnished yellow coloured crystals, re-crystallized from methanol, m.p. 64-68^{0}C,isolated from ethyl acetate:methanol (3:7v/v) fractions of the column. IR (KBr): 3390(N-H band), 1642(Secondary amide carbonyl function), 1565.46(Secondary acyclic amide), 655-734(oop N-H wagging), 1250 cm^{-1} (interaction between NH bending & CN stretching). ^{1}H–NMR (400MH_Z $CDCL_3$): δ1.9(2H, H-1'),1.3 (3H, H-2'), 3.0-3.8(10H, 5xCH_2;5H, 5xCH; 4H, 4OH; H_2 to H-6") 7.8 (1H, NH). ^{13}C-NMR (400MH_Z $CDCL_3$): C-1(178.09), C-2(63), C-3(36), C-4(29), C-5(67), C-1" (79), C-2" (78), C-3" (77),C-4"(74), C-5" (73), C-6" (72),C-1'(36), C-2'(24). UV λ_{max}: 220 nm (in methanol).

DISCUSSION

Compound **A1** was assigned the molecular formula $C_{15}H_{18}O_7$ ([M] $^+$= m/z 310). Its IR spectrum showed the presence of γ-lactone moiety at 1784.52 cm^{-1},a tertiary – OH at 3431 cm^{-1}, carbonyl group of -COOH group at 1727.54 cm^{-1}, methyl & methylene groups at 2922.48 & 2851.54 cm^{-1}, ethylenic double bond is indicated at 1575 cm^{-1}. In the ^{1} H NMR spectrum, singlets at δ1.25 and δ1.79 were attributed to the usual tertiary methyl on C-13 and the vinylic methyl on C-2', in addition two singlets at δ4.91and δ 4.87 represented the C-1'methylene and one oxymethine proton at δ 4.09. Doublet at δ 4.26 represents C-4 methine hydrogen and is downfield due to oxygen. It gets split by the proton at C-5. The ^{13}C NMR spectrumof A1 showed the presence of 15 carbons of which two were carbonyl carbons at δ175.0(C-2) and δ170.0 (C-14). One of them was assigned to a γ-lactone and the other to a carbonyl group carbon. Two oxygenated epoxide carbons at C-10 & C-1 were given values δ 52.3 & δ 47.0. LC-MS m/z (rel. int.): The molecular ion sodiated peak at 333 (310+23) corresponds to the molecular formula. Compound **A2** was assigned the molecular formula $C_{15}H_{18}O_7$ ([M] $^+$ = m/z 310) Its IR spectrum showed the presence of OH group, two γ-lactone rings & C-H bonds at 3448.26, 1787,1731,2963,2933,1646,1439,1305,1116.35,991.35,756.4cm^{-1}. In the^{1}HNMR spectrum singlets at δ 4.9, 3.85, 3.44, 2.9 & 3.72 were attributed to the methine proton at C-8, C-9, C-14 ,C-12, C-3 and two singlets at δ 2.76 and 2.21 represented the methylene group on C-2 and singlet at δ 1.33representedone tertiary methyl group on C-15 .The ^{13} C NMR spectrum of compound A3 showed the presence of 15 carbons two of which are lactone carbonyl carbons at δ 174 & δ 169. Compound **A3** was assigned the molecular formula $C_{16}H_{20}O_7$ ([M] $^+$ = m/z 324). Its IR spectrum showed the presence of a tertiary hydroxyl group at 3378 cm^{-1}, γ-lactone moiety at 1782.6 cm^{-1}, an ester moiety at 1727 cm^{-1}, two C-O groups at 1032 cm-^{1} and at 772 cm^{-1}, one C=C at 1576.6 cm^{-1}. In the ^{1}H NMR, spectrum, singlets at δ1.28 and 1.78 were attributed to tertiary methyl group on C-2 and the vinylic methyl on C-2' resp., in addition two singlets at δ 4.90 and 4.87 represented the C-1'methylene and proton at C-9 is downfield with double doublet a δ3.9.^{13}C NMR spectrum of A3 showed the presence of 16 carbons of which two were carbonyl carbons at δ176.76(C-5) and δ173.65 (C-14). Compound **A4** was assigned the molecular formula $C_{25}H_{47}ON$ ([M] $^+$ = m/z 377). Its IR spectrum showed the presence of N-CH_3 group, NH, C=O,

C=C,CH_3,CH_2(CHsaturated)&CH_3at1166.52,1556.45,1787.50,1729.50,1378.52,1304.51,2926.36and 2854.44 cm^1respectively.The ^{1}H NMR spectrum exhibited singlet at δ7.2686 (NH) & at 4.83(C_5 –CH). The carbonyl carbon of the secondary amide appeared at 167.66. The presence of a conjugated system was confirmed by carbon signals at δ141.8, 132.34, 130.92 & 128.8. Signals from 14.09- 48.07 were suggestive for 18 carbon atoms. The molecular ion is 377 therefore confirms the molecular formula $C_{25}H_{47}ON$. It is found to be long chain aliphatic amide. Compound **A5** was assigned the molecular formula $C_{13}H_{25}O_7$ N ($[M]^+$ = m/z 307. C-13 signal at 178.09 indicates the presence of a carbonyl carbon. Absorption at (1642 cm^{-1}) is of the secondary amide carbonyl group. N-H band at 3390 in IR supports mono substituted secondary amide[18]. N-H bending in IR occurs at 1640-1550cm^{-1} for primary and secondary amides. It was seen at 1565.46 in IR of A5. Relatively strong bending bands at 1550 cm^{-1} are reported for secondary amides. Simple open chain secondary amides absorb near 1640 cm^{-1} in the solid state[19]. Secondary acyclic amides appeared at 1565 cm^{-1}. The mass fragment at 355 accounted for the diprotonated disodiated Molecular ion peak (307 +46+2H= 355). The molecular ion is 307 therefore confirms the molecular formula $C_{13}H_{25}O_7N$.

CONCLUSION

Compound A1, A2 & A3 were isolated and characterized which were also reported by other authors. However, isolation of (2Z, 4Z) -N-methyltetracosa-2, 4-dienamide (Compound A4) & N–ethyl–5–O–*β*–d–glucopyranosyl pentanamide (Compound A5) were reported for the first time.

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