SHORT PAPER

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Four new sesquiterpenoids from the soft coral *Nephthea chabrollii*[†]

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Chemical examination of the soft coral *Nephthea chabrolii* collected from the Mandapam coast in the Gulf of Mannar, afforded nine sesquiterpenoids of which four (**1–4**) are new. The structures of the new compounds (**1–4**) have been established by the interpretation of spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, NOESY and mass) and chemical methods.

The family Nephtheidae comprises many genera which *Lemnalia*, *Paralemnalia*, *Capnella*, *Litophyton* and *Nephthea* have received considerable attention from organic chemists.[1] In our continuing interest on the bioactive secondary metabolites from marine organisms,[2] we have examined the soft coral *Nephthea chabrolii* collected from the Mandapam coast in the Gulf of Mannar during October 1997. A literature survey revealed that the genus *Nephthea* has afforded sesquiterpenes derived from germacrene,[3] guaiane,[4] cadinane[5] and caryphyllines[6] skeletons and polyhydroxy steroids.[7–15] The 1:1 dichloromethane:methanol extract of the soft coral *Nephthea chabrolii* was subjected to gel filtration (Sephadex LH-20) followed by silica gel chromatography to afford nine sesquiterpenoids.

Compound **1** was obtained as colourless oil, $[\alpha]^{25}_D$ +9° (*c* 0.2, $CHCl_3$) and was assigned the molecular formula $C_{16}H_{28}O_2$ in combination with high resolution mass measurements at *m/z* 220 (M^+-CH_3OH) and ^{13}C NMR spectral data. The ^{1}H NMR ($CDCl_3$) spectrum of compound **1** showed signals for the presence of a trisubstituted double bond proton at δ 5.46 (1H, s), a methoxyl group at δ 3.16 (3H, s), two tertiary methyls connected to oxygen bearing carbons at δ 1.17 (3H, s) and 1.19 (3H, s) and an isopropyl group at δ 0.97 (3H, d, *J* = 6 Hz) and 0.96 (3H, d, *J* = 6 Hz).[16] The foregoing spectral data was found to be similar to that of guaian-6-en-4, 10-diol,[4] previously isolated from gorgonian coral *Lemnalia africana*,[17] except for an additional signal for a methoxyl group at δ 3.16 (3H, s) (δ C 49.9 q), which could be placed either at C-4 or C-10. The disposition and relative stereochemistry of methoxyl group was established by ^{1}H–^{1}H–COSY and NOESY correlations. The ^{1}H NMR spectrum in C_5D_5N of compound **1** showed good resolution particularly in respect of two tertiary methyls connected to oxygen bearing carbon at δ 1.18 (3H, s, H_3- 11) and 1.38 (3H, 32, H_3 – 15). In the ^{1}H–^{1}H COSY spectrum (d_5 – pyridine) of compound **1**, the trisubstituted double bond proton at δ 5.88 (1H, d, *J* = 2.7 Hz, H-6) showed correlation with methine proton at δ 1.87 (1H, m, H-5), which in turn correlates with δ 1.80 (1H, m, H-1). In the NOESY spectrum the vinylic proton at δ 5.88 (1H, d, *J* = 2.2 Hz) showed strong correlations with isopropyl group δ 0.97 (6H, d, *J* = 7 Hz), 2.42 (1H, m, H-12) and to a quaternary methyl at δ 1.38 (3H, s, H_3-15). Further, the methoxyl group at δ 3.15 (3H, s) showed correlations with the methine proton at δ 1.80 (1H, m, H-1) and to a quaternary methyl at δ 1.18 (3H, s, H_3-11). Further, the presence of a methoxyl group at C-10 was confirmed as follows. Compound **1** on treatment with *m*-chloroperbenzoic acid afforded mono eposide (**5**) without any ether bridge formation between C-10 and C-7 carbons derived from transannular attack of *trans* C-10 alcohol and the nascent epoxide between C-6 and C-7 as noticed in guaian-60-en-4, 10-diol.[4] The coupling constant between H-5 and H-6 protons in 5 (*J* - 6.5 Hz) favoured the anti as noticed in forthcoming compound (**2**) rather than gauche relationship. From foregoing spectral data the structure of compound (**1**) was established as 10α-methoxy-4β-hydroxy guaian-6-ene. Compound **1** may be an artifact obtained during extraction procedure.

Compound **2** was obtained as a viscous liquid, $[\alpha]_D{}^{25}$ +8° (*c* 0.2, $CHCl_3$). It showed no absorptions in UV light and had a molecular ion at *m/z* 218 (M^+-H_2O) and analysed for $C_{15}H_{24}O_2$ by HREIMS, requiring four degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of compound **2** contained signals for an exocyclic methylene at δ 4.68 (2H, brs) (δ_c 151.59 s and 107.25 t), a trisubstituted epoxy group at δ 2.88 (1H, d, *J* = 6.5 Hz) (δ_c 64.96 and 62.14 s), a tertiary methyl connected to an oxygen bearing carbon at δ 1.41 (δ_c 26.22 q, 80.17 s) and an isopropyl group at δ 0.98 (3H, d, *J* = 6.5 Hz), 0.92 (3H, d, *J* = 6.5 Hz) (δ_c 17.69 q and 18.64 q). These data implied that compound **2** is tricyclic in nature and reminiscent of guaian-6, 10-dien-04-ol [4] except for the presency of epoxy group instead of double bond between C-6 and C-7 . To confirm the structure of compound **2**, guaian-6, 10-dien-4-ol was reacted with *m*-chloro perbenzoic acid to yield mono epoxy compound **2** and diepoxy compound **6** in 3:1 ratio. The synthetic sample of **2** was found to be identical (TLC, ^{1}H NMR and mass) to the natural product **2**, establishing that the epoxy group is situated between C-6 and C-7. The doublet (*J* = 6.5 Hz) nature of epoxy methine proton at δ 2.88 indicated that oxirane ring is in β orientation[4] and was further confirmed by NOESY spectrum. The epoxy methine proton at δ 2.88 showed correlations with C-4-methyl at δ 1.41 and one of the isopropyl methyl at δ 0.92. The structure of compound (**2**) was thus established as 6β, 7β-epoxy-4β-hydroxy guaian-10-ene.

Compound **6** was obtained as solid, mp 75-77 °C, $[\alpha]^{25}_D$ + 16.3° (*c* 0.2, $CHCl_3$). The ^{1}H NMR spectrum of compound **6** is similar to that of compound **2** except for the disappearance of exocyclic methylene protons at δ 4.70 (2H, brs), instead, two doublets at δ 2.68 (1H, d, *J* = 5.5 Hz) and 2.48 (1H, d, *J* = 5.5 Hz) were observed. Further, the C-6 epoxide methine proton appeared as singlet at δ 3.0 (1H, s) indicated the α orientation of epoxide ring.[3]

Compound **3** was obtained as liquid $[\alpha]^{25}_D$ + 2.8° (*c*, 0.5, $CHCl_3$) showed no UV absorptions beyond 200 nm, and analysed for $C_{15}H_{24}$, *m/z* 204 (M^+) by HR EIMS requiring four degrees of unsaturation. The ^{1}H NMR spectrum of

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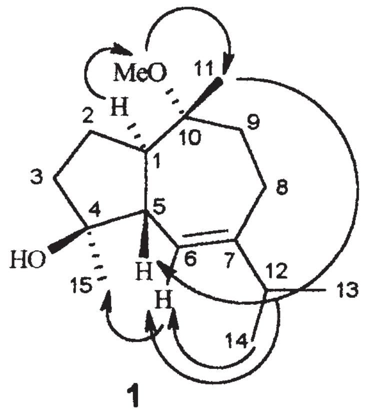
[†] This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



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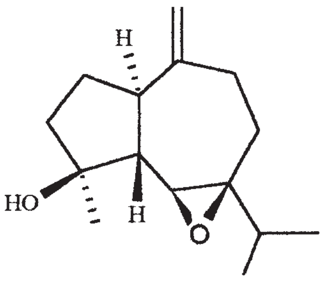
Arrows denotes NOESY correlations



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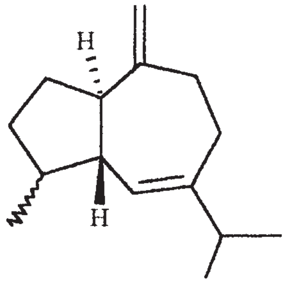

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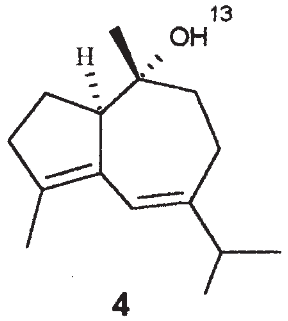

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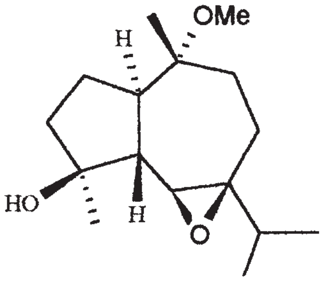

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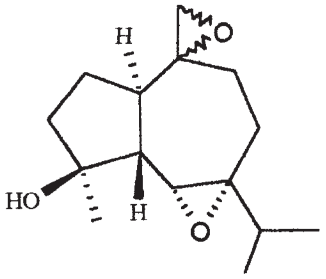

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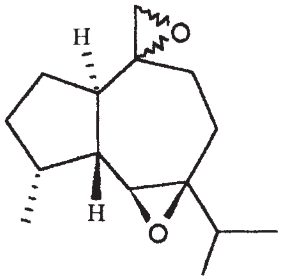

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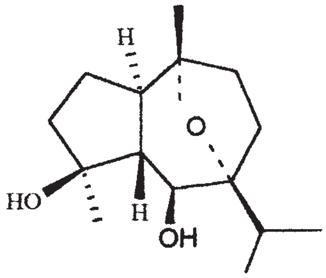

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compound **3** contained signals for a trisubstituted double bond at δ 5.63 (1H, brs), an exocyclic methylene proton at δ 4.82 (2H, brs), an isopropyl group at δ 1.0 (6H, d, $J = 7.0$ Hz) and a secondary methyl at 0.92 (3H, d, $J = 6.5$ Hz). The presence of two double bonds was further confirmed by the ^{13}C NMR spectrum (δ_c 154.73 s, 148.46 s, 123.92 d and 106.04 t). The ^1H and ^{13}C NMR spectral data indicated that compound **3** is a guaiane based sesquiterpene.[4]. To correlate compound **3** with guaiane skeleton, compound **3** was treated with m-chloroperbenzoic acid to afford a diepoxy compound **7**. The chemical shift and the coupling constant of the epoxy methine proton (C-6) in compound **7** was found to be exactly matching [δ 2.88 (1H, d, $J = 6.5$ Hz)] with compound **2**. Since compound **3** showed no UV absorptions beyond 200 nm and the absence of doubly allylic methine proton in its ^1H NMR spectrum, the structure of compound (**3**) was established as 4,15-dihydro guaia-6, 10-diene.

Compound **4** was obtained as an oil, $[\alpha]^{25}_D$ +3° (*c* 0.2, $CHCl_3$), UV (MeOH) 236 (15 000) and showed molecular ion at *m/z* 220, analysed for $C_{15}H_{24}O$ by HR EIMS. The IR spectrum showed bands for the presence of hydroxyl (3500 cm^{-1}) and double bond (1620 cm^{-1}) functionalities. The ^1H NMR spectrum of compound **4** showed signals for the presence of α,β-unsaturated trisubstituted double bond proton at δ 6.25 (1H, brs), a vinylic methyl at δ 1.8 (3H, s), a tertiary methyl connected to an oxygen bearing carbon at δ 1.24 (3H, s) and an isopropyl group at δ 1.1 (6H, d, $J = 6.8$ Hz). The foregoing spectral data and a literature survey revealed that compound **4** is closely related to a sesquiterpene-4, 6-guaiadiene[18] except for the presence of hydroxyl group at C-10. Thus the structure of compound (**4**) was assigned as guaian-4, 6-dien-10α-ol.

The known compounds guaian-6, 10-dien-4-ol,[4] guaian-6-en-4,10-diol,[4] β-elemene,[19] δ-elemene,[20] germacrene-C[21] and compound **8**[4] were characterized by comparing the physicospectral data with those reported in literature. The naturally occurring compound (**8**) showed small optical rotation $[\alpha]^{25}_D$ + 0.88 (*c* 0.7, $CHCl_3$) and was confirmed by treating guaian-6-en-4β,10α-diol[4] with *m*-chloroperbenzoic acid to obtain compound **8** which showed no optical activity. Compound **8** may an artifact obtained during isolation procedures.

Experimental

The ^1H and ^{13}C NMR (50 MHz) spectra were recorded on a Varian Gemini 200 MHz spectrometer using TMS as internal standard. Chemical shifts are reported in parts per million, and coupling constants (*J*) are expressed in Hertz. UV and IR spectra were recorded on Shimadzu 240 and Perkin-Elmer 1310 spectrophotometers respectively. Mass spectra were recorded on a Finnigan-MAT 1020 instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Collection extraction and isolation procedure: The soft coral *Nephthea chabrolii* Andoum (Nephtheidae) (IIC-266) was collected at Mandapam coast (N 9°18^1, E 79°08^1) in the Gulf of Mannar by skin diving at 20 feet depth during October 1997. The voucher specimen (IIC-266) is on deposit at the National Institute of Oceanography, Goa, India. The freshly collected (2.2 kg wet weight) specimen was cut into pieces and soaked in MeOH until workup. The aqueous MeOH from the organism was decanted and the coral was freeze-dried. The freeze-dried material was extracted with CH_2Cl_2–MeOH (1:1, 3 × 2.5 l). The combined extracts were concentrated under reduced pressure and the crude extract (50 g) was partitioned between H_2O and EtOAc. Concentration of the organic layer afforded a brownish gummy crude extract (30 g). It was subjected to gel filtration (Sephadex, LH-20) followed by silica gel (100–200 mesh) column chromatography using hexane through hexane-ethyl acetate mixtures to methanol as eluents. The fraction eluting with hexane afforded compound **3**, 2% EtOAc – hexane afforded β-elemene and δ-elemene, 5% EtOAc-hexane fraction yielded compounds **4**, germacrene-C and 10% EtOAc–hexane fraction afforded compounds **2** and guaian-6,10-dien-4-ol. Compound **1** was obtained from the 15% EtOAc-hexane fraction and guaian-6-en-4, 10-diol was isolated from the 25% EtOAc-hexane fraction as a solid.

10α-Methoxy-4β-hydroxy guaian-6-ene (**1**): viscous liquid (80 mg), $[\alpha]^{25}_{D}$ +9° (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$); δ 5.46 (1H, s), 3.15 (3H, s), 2.45 (1H, m), 2.22–2.08 (5H, m), 1.52 (5H, m), 1.19 (3H, s), 1.17 (3H, s), 0.97 (3H, d, $J = 6$ Hz), 0.96 (3H, d, $J = 5$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$); δ 149.07 (s), 121.37 (d), 79.82 (s), 79.11 (s), 49.9 (q), 48.47 (d), 47.76 (d), 40.35 (d), 37.05 (t), 35.47 (t), 24.45 (t), 22.25 (t), 21.58 (q), 21.43 (q), 21.12 (q) and 17.86 (q); EI MS: *m/z* 220 (M-CH_3OH, 3)$^+$, 162 (17), 147 (8); HREIMS (*m/z*): 220.1819 (M-CH_3OH)$^+$ for $C_{15}H_{24}O$ for 220.1827.

^1H NMR (400 MHz, C_5D_5N): δ 5.88 (1H, d, $J = 7.2$ Hz), 3.15 (3H, s), 1.60–1.90 (7H, m), 2.21–2.27 (3H, m), 2.59 (1H, m), 1.38 (3H, s), 1.18 (3H, s) and 0.97 (6H, d, $J = 7$ Hz).

6β, 7β-Epoxy-4α-hydroxy guaian-10-ene (**2**): colourless viscous liquid (150 mg), $[\alpha]^{25}_{D}$ +8° (*c* 0.2, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$); δ 4.68 (2H, brs), 2.88 (1H, d, $J = 6.5$ Hz), 2.42–2.24 (2H, m), 2.18–2.03 (3H, m), 1.88–1.72 (3H, m), 1.7–1.5 (3H, m), 1.41 (3H, s), 0.98 (3H, d, $J = 6.5$ Hz) and 0.92 (3H, d, $J = 6.5$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$); δ 151.59 (s), 107.25 (t), 80.17 (s), 64.96 (s), 62.14 (d), 57.41 (d), 47.78 (d), 41.38 (d), 34.54 (t), 33.95 (t), 29.27 (t), 26.22 (t), 24.87 (q), 18.64(q) and 17.69 (q); EI MS : *m/z* (M-H_2O)$^+$; HREIMS (*m/z*): 218.16826 (M-H_2O)$^+$ for $C_{15}H_{22}O$, for 218.16706.

4,15-Dihydro guaian-6, 10-diene (**3**): was obtained as a pale yellow liquid (150 mg), $[\alpha]^{25}_{D}$ +2.8° (*c* 0.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$); δ 5.63 (1H, brs), 4.82 (2H, brs), 2.52 (1H, m), 2.32 (2H, m), 2.25–2.0 (5H, m), 1.75 (2H, m), 1.25 (2H, m), 1.0 (6H, d, $J = 7$ Hz) and 0.92 (3H, d, $J = 6.5$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 154.73 (s), 148.46 (s), 123.92 (d), 106.04 (t), 47.7 (d), 37.41 (d), 36.83 (d), 32.88 (t), 30.06 (t), 27.67 (t), 21.49 (q), 21.31 (q) and 17.07 (q); EI MS : *m/z*: 204 (M)$^+$; HREIMS (*m/z*) 204.1876 (M)$^+$ for $C_{15}H_{24}$, for 204.1878.

Guaian-4, 6-dien-10α-ol (**4**): was obtained as a colourless oil (5 mg), $[\alpha]^{25}_{D}$ +3° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 236 (15,000): IR (neat): ν_{max} 3500, 1620 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$); δ 6.25 (1H, brs), 2.60 (2H, m), 2.45–2.20 (5H, m), 1.8 (3H, s), 1.75 (1H, m), 1.52 (2H, m), 1.24 (3H, s) and 1.1 (6H, d, $J = 6.8$ Hz); EI MS : *m/z* : 220 (M)$^+$; HREIMS (*m/z*): 220.1704 (M)$^+$ for $C_{15}H_{24}O$ for 220.17042.

Epoxidation of 10α-Methoxy-4β-hydroxy guaian-6-ene (**1**): To an ice cold dichloromethane solution (10 ml) of 10-α-methoxy-4β-hydroxy guaian-6-ene (20 mg), *m*-chloroperbenzoic acid (30 mg) was added and the reaction mixture was stirred for 30 minutes. After usual workup, the reaction mixture was chromatographed over silica gel to give compound (**5**).

6β, 7β-Epoxy-10α-methoxy-4β-hydroxy guaiane (**5**): colourless liquid, $[\alpha]^{25}_{D}$ +5.8° (*c* 0.6, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 3.16 (3H, s), 2.88 (1H, d, $J = 6.5$ Hz), 2.12–1.75 (5H, m), 1.65–1.40 (7H, m), 1.35 (3H, s), 1.12 (3H, s) and 1.0 (6H, d, $J = 6.0$ Hz); EI MS: *m/z* 236 (M-CH_3OH)$^+$.

Epoxidation of guaian-6, 10-dien-4α-ol : To an ice cold dichloromethane solution (10 ml) of guaian-6,10-dien-4-ol (30 mg), *m*-chloroperbenzoic acid (35 mg) was added and the reaction mixture was stirred for 30 minutes. After usual workup, the reaction mixture was chromatographed over silica gel to afford mono epoxide (**2**) and diepoxide (**6**).

6α, 7α and 10,11-diepoxy-4β-hydroxy guaiane (**6**): white solid, mp 75–77 °C; $[\alpha]^{25}_{D}$ +16.3° (*c* 0.2, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 3.03 (1H, s), 2.68 (1H, d, $J = 5.5$ Hz), 2.48 (1H, d, $J = 5.5$ Hz), 2.32 (2H, m), 2.05–1.9 (4H, m), 1.75–1.45 (6H, m), 1.32 (3H, s), 0.98 (3H, d, $J = 7.0$ Hz), 0.92 (3H, d, $J = 7.0$ Hz); EI MS : *m/z* 209 (M–C_3H_7)$^+$.

Epoxidation of 4,15-dihydro guaian-6,10-diene (**3**): To an ice cold dichloromethane solution (10 ml) of 4,15-dihydro guaian-6,10-diene (30 mg), *m*-chloroperbenzoic acid (40 mg) was added and the reaction mixture was stirred for 30 minutes. After usual workup, the reaction mixture was chromatographed over silica gel to afford a diepoxide (**7**).

6β, 7β and 10,11-diepoxy guaiane (**7**): white solid 58–60 °C; $[\alpha]^{25}_{D}$ +4.6° (*c* 0.3, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 2.88 (111, d, $J = 6.5$ Hz), 2.59 (1H, m), 2.38 (1H, d, $J = 4.5$ Hz), 2.35–2.12 (3H, m), 2.02–1.85 (2H, m), 1.75–1.45 (4H, m), 1.38 (3H, s), 1,02 (3H, d, $J = 6.5$ Hz), 0.92 (3H, d, $J = 6.5$ Hz), 0.92 (3H, d, $J = 6.5$ Hz); EI MS : *m/z* 193 (M-C_3H_7)$^+$.

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