SHORT REPORT

A C G publications

Rec. Nat. Prod. 7 :2 (2013) 141-146

records of natural products

Melicimides A and B: Two New Ceramides from Stem Bark of *Melicia excelsa*

Hidayat Hussain[*1,2], Awazi Tengu Nyongha[3], Etienne Dongo[*3], Amin Badshah[4], Ivan R. Green[5] and Wen Zhang[*6]

[1]*Department of Chemistry, Universität Paderborn, Warburger Straße 100, 33098 Paderborn, Germany*
[2]*Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Birkat Al-Mouz, Nizwa 616, Sultanate of Oman*
[3]*Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P. O. Box 812, Yaounde, Cameroon*
[4]*Department of Chemistry, Quaid-i-Azam University, 45320 Islamabad, Pakistan*
[5]*Department of Chemistry, University of Stellenbosch, P/Bag X 1, Stellenbosch,7600, South Africa*
[6]*Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, 325 Guo-He Road, Shanghai 200433 P. R. China*

(Received November 26, 2012; Revised January 16x, 2013; Accepted January 18, 2013)

Abstract: Melicimides A (**1**) and B (**2**), two new ceremides have been isolated from *Melicia excelsa* (Moraceae). The structures of the new compounds were determined by comprehensive analyses of their 1D (^{1}H and ^{13}C NMR), 2D NMR (including COSY, HMQC, and HMBC) and ESIMS spectral data. A known compound viz., β-sitosterol has also been identified.

Keywords: Moraceae; *Melicia excelsa*; Ceramide; Structure elucidation.

1. Plant Source

In the course of phytochemical studies of medicinal plants from Africa [1-6], we investigated *Melicia excelsa* (Moraceae). We now report on the identification of two new ceramides which we have named melicimides A (**1**) and B (**2**) (Figure 1).
The stem bark of the plant *M. excelsa* was collected from its natural habitat in Yaounde, Cameroon in March 2005 and March 2010, and identified by Dr Tsabang Nole (plant taxonomist) at the Ministry of Scientific Research, Yaounde. A specimen of this plant (N^{o} HNC 57226) is deposited at the Cameroon National Hebarium (Yaoundé).

* Corresponding author: E- Mail:Hidayat110@gmail.com, dongoet@yahoo.fr

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 3/15/2013 EISSN: 1307-6167

2. Previous Studies

One cermamide has been isolated from *M. excelsa* [7].

3. Present Study

The air-dried leaves of *M. excelsa* (5 kg) were ground and extracted with EtOAc to afford a crude extract (120 g) which was subsequently subjected to liquid column chromatography over silica gel (Merck, 230-400 mesh) eluting with *n*-hexane, *n*-hexane/EtOAc, EtOAc and EtOAc/MeOH, in increasing order of polarity. The fraction F_2 (0.5 g) was subjected to gradient column chromatography using acetone and *n*-hexane (5% acetone:*n*-hexane) as eluent to afford a white crystalline solid of β-sitosterol (11.5 mg). Repeated column chromatography of fraction F_5 (1.3 g) by gradient elution using *n*-hexane and acetone (50% acetone:*n*-hexane) as eluent yielded a white solid identified as melicimide A (**1**, 10.5 mg). Fraction F_9 (1.6 g) was also reloaded onto a second column of silica gel and eluted with acetone:*n*-hexane (7:3) to give a white solid identified as melicimide B (**2**, 7.0 mg).

Melicimide A (1): M. p. 150 °C; white solid. $[\alpha]_D^{20}$ + 10.01º (*c* = 0.91, $CHCl_3$ + MeOH); IR ν_{max} ($CHCl_3$ + MeOH): 3610, 2935, 2865, 1640, 1290 cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ 0.85 (t, *J* = 6.5 Hz, 6H, H–18', H–26), 1.24 (bs, 66H, H-7-25, H-4'-17'), 1.69 (m, 2H, H–3'), 1.77 (m, 2H, H–6), 2.02 (m, 2H, H–5), 4.27 (m, 1H, H–4), 4.34 (dd, *J* = 4.5, 6.5 Hz, 1H, H–3), 4.41 (dd, *J* = 4.5, 10.5 Hz, H–1b), 4.51 (dd, *J* = 4.5, 10.5 Hz, 1 H, H–1a), 4.62 (m, 1H, H–2'), 5.10 (m, 1H, H–2), 8.53 (d, *J* = 8.8 Hz, 1H, NH). ^{13}C-NMR (150 MHz, C_5D_5N): δ 14.6 (C-18', C-26), 23.3 (C-24), 26.2 (C-17'), (C-25), 27.1 (C-16'), 30.5 (C-7-23, C-4'-15'), 32.5 (C-6), 33.3 (C-5), 34.2 (C-3'), 53.3 (C-2), 62.4 (C-1), 72.8 (C-2'), 73.3 (C-4), 77.1 (C-3), 175.6 (C-1'). EIMS: See Figure 2; Negative mode EISMS: *m/z* 710.6655 [M^+-H] (Calcd for $C_{44}H_{88}NO_5$, 710.6662).

Melicimide B (2): White solid: $[\alpha]_D^{20}$ + 10.2º (*c* = 0.91, $CHCl_3$ + MeOH); IR ν_{max} ($CHCl_3$): 3405, 2865, 1635, 1535, 1290 cm^{-1}. ^{1}H-NMR (600 MHz, C_5D_5N): δ 0.89 (t, *J* = 6.5 Hz, 6H, H–18', H–23), 1.29 (s, 50H, H-7-16, H-21, H-22, 5'-17'-H), 1.68 (m, 2H, H–4'), 1.87 (m, 2H, H–6), 2.08 (m, 2H, H–5), 2.11 (m, 2H, H-20), 2.17 (m, 2H, H-17), 4.34 (m, 1H, H–4), 4.37 (m, 1H, H–3), 4.59 (m, 2H, H–3′), 4.47 (dd,1H, *J* = 4.5, 10.5 Hz, H–1b), 4.52 (dd, 1H, *J* = 4.5, 10.5 Hz, H–1a), 4.59 (m, 2H, H–3′), 4.80 (m, 1H, H–2′), 5.17 (m, 1H, H–2), 5.55 (dt, 1H, *J* = 14.0, 6.0 Hz, H-19), 5.60 (dt, 1H, *J* = 14.0, 6.0 Hz, H-18), 8.53 (d, 1H, *J* = 8.8 Hz, NH). ^{13}C-NMR (150 MHz, C_5D_5N): δ 14.7 (C-18', C-23), 23.3 (C-22), 27.0 (C-17'), 27.1 (C-15'), 30.5 (C-7-16, C-21, C-5'-14'), 32.5 (C-6), 33.0 (C-5, C-4'), 33.4 (C-20), 33.7 (C-17), 53.5 (C-2), 62.3 (C-1), 73.2 (C-4), 74.1 (C-3′), 76.8 (C-2′), 77.2 (C-3), 131.2 (C-18), 131.1 (C-19), 174.3 (C-1'). EIMS: See Figure 3; ESIMS: 706.5949 [M^++Na] (Cald. for $C_{41}H_{81}NO_6Na$, 706.5962)

Melicimide A (**1**) (Figure 1) was isolated as a white powder, $[\alpha]_D^{20}$ + 10.01º. The molecular formula ($C_{44}H_{89}NO_5$) of **1** was determined through ESIMS.

A ceramide skeleton was supported by the presence of the following signals in the ^{1}H-NMR spectrum: δ 0.85 (t, 6H, *J* = 6.8 Hz, for H–26 and H–18′) representing the two terminal methyl groups. A sharp but broad signal at δ 1.24 integrating for 66H is indicative of the two long methylene side chains while an amide proton at δ 8.53 (d, *J* = 8.0 Hz supported an amide functionality. This was further supported by an NH-attached to a methine carbon signal at δ 53.3 (C-2) and a signal at δ 175.7 for a C=O carbon in the ^{13}C NMR spectrum [1-6]. In addition the IR spectra suggested the presence of secondary amide group due to a strong absorption band at 1640 cm^{-1}) [1-6]. The ^{1}H NMR spectrum in pyridine-d_5 exhibited three oxymethine protons: a) δ 4.62 (m, H–2′), b) 4.34 (dd, *J* = 4.5, 6.5 Hz, H–3), and c) 4.27 (m, H–4)] as well as two oxymethylene proton signals at δ 4.51 (dd, *J* = 4.5, 10.5 Hz, H–1a) and 4.41 (dd, *J* = 4.5, 10.5 Hz, H–1b). The presence of four hydroxyl groups was further confirmed by the presence of four oxygenated carbons at δ 77.1 (d), 73.3 (d), 72.8 (d), and 62.4 (t) in

the ^{13}C NMR spectrum. The presence of the hydroxyl groups was further supported by the absorption band at 3610 cm^{-1} in the IR spectrum [1-6].

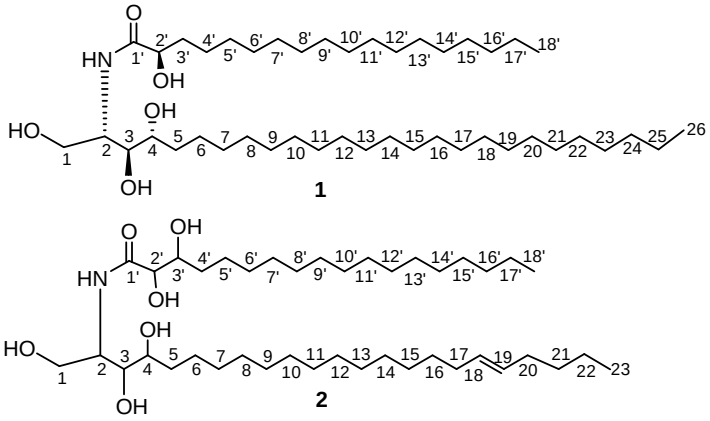

Figure 1. Structures of melicimides A (**1**) and B (**2**).

A ceramide skeleton was supported by the presence of the following signals in the ^{1}H-NMR spectrum: δ 0.85 (t, 6H, *J* = 6.8 Hz, for H–26 and H–18′) representing the two terminal methyl groups. A sharp but broad signal at δ 1.24 integrating for 66H is indicative of the two long methylene side chains while an amide proton at δ 8.53 (d, *J* = 8.0 Hz supported an amide functionality. This was further supported by an NH-attached to a methine carbon signal at δ 53.3 (C-2) and a signal at δ 175.7 for a C=O carbon in the ^{13}C NMR spectrum [1-6]. In addition the IR spectra suggested the presence of secondary amide group due to a strong absorption band at 1640 cm^{-1}) [1-6]. The ^{1}H NMR spectrum in pyridine-d_5 exhibited three oxymethine protons: a) δ 4.62 (m, H–2′), b) 4.34 (dd, *J* = 4.5, 6.5 Hz, H–3), and c) 4.27 (m, H–4)] as well as two oxymethylene proton signals at δ 4.51 (dd, *J* = 4.5, 10.5 Hz, H–1a) and 4.41 (dd, *J* = 4.5, 10.5 Hz, H–1b). The presence of four hydroxyl groups was further confirmed by the presence of four oxygenated carbons at δ 77.1 (d), 73.3 (d), 72.8 (d), and 62.4 (t) in the ^{13}C NMR spectrum. The presence of the hydroxyl groups was further supported by the absorption band at 3610 cm^{-1} in the IR spectrum [1-6].

The ^{1}H NMR spectrum of **1** displayed an additional signal at δ 5.10 assigned to H-2 and methylene proton signals were found to be overlapped in the range δ 1.29-2.20. The ^{13}C NMR showed signals for several methylene groups in the range of δ 26.2-34.2, and the terminal methyl groups of the aliphatic chains at δ 14.7 indicated the absence of any branching [1-9]. The position of the hydroxyl groups was confirmed by the mass fragmentation pattern (Figure 2b), the ^{1}H-^{1}H-COSY, and the HMBC spectra (Figure 2a). Cross peaks in ^{1}H-^{1}H-COSY were observed between the amide proton (δ 8.53) and the (H–2) methine proton (δ 5.11), which, in turn, was coupled to three protons at δ 4.51 (H–1a), δ 4.41 (H–1b), and δ 4.34 (H–3). Additionally, H–1a showed the expected correlations with H-2 and no cross peaks were observed between the signal at δ 4.62 assigned to H-2′ to any downfield proton signals the latter only showing correlation to upfield signals. In the HMBC spectrum the signal at δ 4.62 showed a strong correlation to C-1′. These results confirmed that the fourth hydroxyl group is present at C-2′ of the fatty acid chain. The position of the other three hydroxyl groups in the long chain amino alcohol was further confirmed from the HMBC correlations (Figure 2a) as well as from the mass fragmentation pattern (Figure 2b)

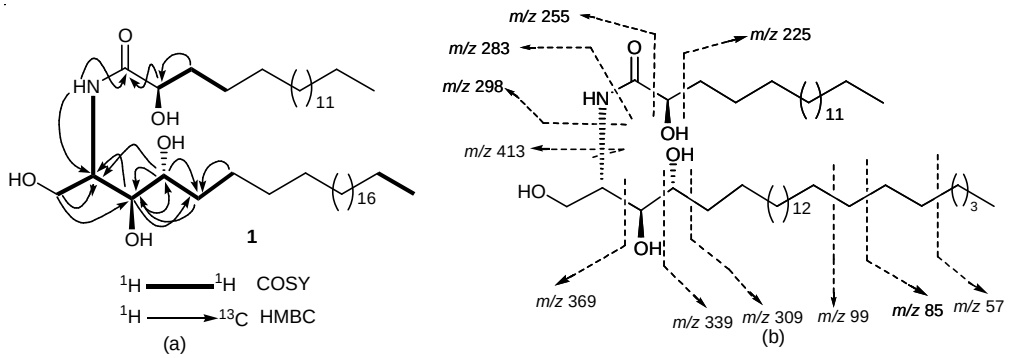

Figure 2. (a) Important ^{1}H-^{1}H-COSY and HMBC correlations for melicimide (**1**) A; (b) Mass fragmentation pattern of compound melicimide A (**1**)

The chain length of the fatty acid moiety was determined from the characteristic fragmentation ions (Figure 2) at *m/z* 283 $[CH_3(CH_2)_{15}CH(OH)CO]^+$, 300 $[CH_3(CH_2)_{16}CH(OH)CONH_2+H]^+$ and 355 $[CH_3(CH_2)_{15}CH(OH)C(OH)=NC(=CH_2)CH_2OH]^+$ in the EIMS. The length of the long chain amino base was also determined by the characteristic fragmentation ions at *m/z* 342 [M-$CH_3(CH_2)_{21}(CHOH)_2]^+$, 369 $[CH_3(CH_2)_{21}(CHOH)_2]^+$ and 386 $[CH_3(CH_2)_{21}(CHOH)_2OH]^+$ in the EIMS [1-7]. The assignments were further confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC correlations (Figure 2a). Thus, the long chain amino base and fatty acid of **1** are assigned as 2-amino-docosane-1,3,4-triol and 2-hydroxyoctadecanoic acid, respectively.

In addition ^{1}H-NMR spectrum of compound **1** corresponded very well to that of the synthetic ceramide (2*S*,2′*R*,3*S*,4*R*)-2-(2-hydroxytetracosanoylamino)hexadecane-1,3,4-triol, with respect to the signals due to H–1a, H–1b, H–2, H–3, H–4, and (H–2′) (Table 1) [8]. The NMR data and comparison of the optical rotation of compound **1** (+10.01) and the synthetic ceramide (+9.1) [8] as well as with related naturally occurring ceramides [6,9], suggested that compound **1** has the same absolute configuration for the core structure in the 2,3,4,2′ part.

Table 1. ^{1}H NMR data (δ and *J* values) of compound **1** and synthetic ceramide in C_5D_5N

H	*Melicimide A (1)*	*Synthetic ceramide*[a]
H-1a	4.51 (dd, *J* = 4.5, 10.5 Hz)	4.52 (dd, *J* = 4.5, 10.7 Hz)
H-1b	4.41 (dd, *J* = 4.5, 10.5 Hz)	4.43 (dd, *J* = 5.0, 10.6 Hz)
H-2	5.10 (m)	5.12 (m)
H-3	4.34 (dd, *J* = 4.5, 6.5 Hz)	4.36 (dd, *J* = 4.6, 6.6 Hz)
H-4	4.27 (m)	4.29 (m)
H-2′	4.62 (m)	4.63 (dd, *J* = 4.0, 7.6 Hz)

[a] Data from ref. [8]

On the basis of this evidence, the structure of **1** is suggested to be (2*S*, 2′*R*, 3*S*, 4*R*)-*N*-[2′-hydroxyoctadecanoyl]-2-amino-docosane-1,3,4-triol.

Melicimide B (**2**) (Figure 1) was isolated as white solid whose molecular formula was established as $C_{41}H_{81}NO_6$ through ESIMS. The ^{1}H NMR spectrum exhibited a doublet at δ 8.53 (*J* = 8.5 Hz) due to an NH proton, a broad single peak at δ 1.29 (methylene protons), a 6-proton triplet at δ 0.89 (two terminal methyl groups) all of which suggests the ceramide nature of the molecule [1-9]. The typical IR absorptions at 1635 and 1535 cm^{-1} suggested an amide linkage, which was confirmed by a nitrogen-attached carbon signal at δ 53.5 and a carbonyl signal at δ 174.3 in the ^{13}C NMR spectrum.

The ^{1}H NMR spectrum of **2** displayed four characteristic signals of protons geminal to hydroxyl groups at δ 4.80 (m, H–2′), 4.59 (m, H–3′), 4.37 (m, H–3), and 4.34 (m, H–4). There were also two oxymethylene proton signals at δ 4.52 (dd, *J* = 4.5, 10.5 Hz, H–1a) and 4.47 (dd, *J* = 4.5, 10.5 Hz, H–1b). This assignment was further supported by four methine signals at δ 77.2 (C-3), 76.8 (C–2′), 74.1 (C–3′), 73.2 (C-4) and one oxymethylene signal at δ 62.3 in the ^{13}C NMR spectrum. The

presence of hydroxyl groups was further confirmed from an IR absorption band at 3405 cm^{-1}. The ^{1}H and ^{13}C NMR spectra furthermore indicated two olefinic proton signals at δ 5.60 (dt, *J* = 14.0, 6.0 Hz, H-18; δ_C 131.2) and δ 5.55 (dt, *J* = 14.0, 6.0 Hz, H-19; δ_C 131.1), attributable to the presence of one *trans* disubstituted double bond. The large vicinal coupling constant between H-18 and H-19 (*J* = 14.0 Hz) in ^{1}H NMR spectrum clearly indicated an *E*-geometry for the double bond. The chain length of the fatty acid was determined by the characteristic fragmentation ions (Figure 3) at *m/z* 271 $[CH_3(CH_2)_{14}(CHOH)_2CO]^+$, 241 $[CH_3(CH_2)_{14}(CHOH)CO]^+$, and 316 $[CH_3(CH_2)_{14}(CHOH)_2CONH_2+H]^+$ in the EIMS. The length of the long chain amino base was also determined by the characteristic fragmentation ions at *m/z* 358 $[M\text{-}CH_3(CH_2)_{16}CH{=}CH(CHOH)_2]^+$, 325 $[CH_3(CH_2)_{16}CH{=}CH(CHOH)_2]^+$ and 342 $[CH_3(CH_2)_{16}CH{=}CH(CHOH)_2OH]^+$ in the EIMS [1-5].

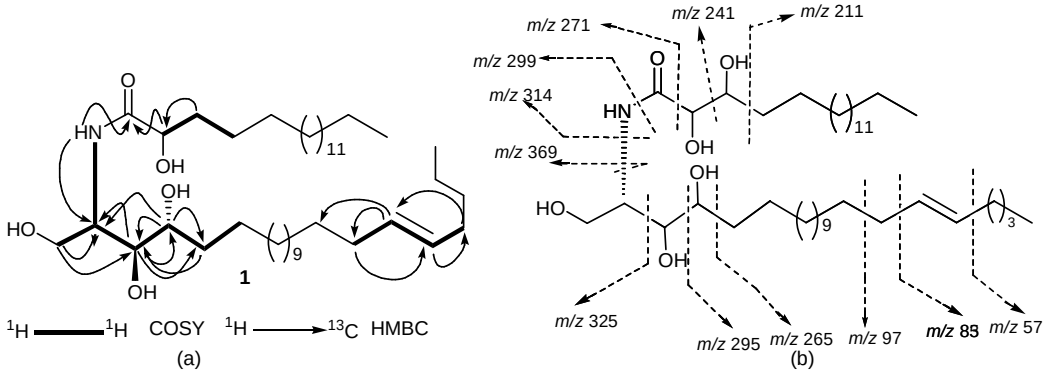

Figure 3. (a) Important ^{1}H-^{1}H-COSY and HMBC correlations for melicimide B (**2**); (b) Mass fragmentation pattern of compound melicimide B (**2**).

Cross peaks in ^{1}H ^{1}H COSY between the amide proton (NH) and H–2, the latter which in turn, showed coupling with H–1a, H–1b, and H–3 suggested this structural feature was in place. Additionally, H–1a showed the expected correlations with H-2 (Figure 3a). Similarly H-2′ showed COSY correlations with H-3′ and in the HMBC spectrum H-2′ and H-3′ showed a strong correlation to C-1′. These results confirmed that four of the five hydroxyl groups were present at C-3, C-4, C-2′, and C-3′. The position of these four hydroxyl groups was further confirmed from the HMBC correlations (Figure 3a) as well as from the mass fragmentation pattern (Figure 3b). The stereochemistries at C-2, C-3, C-4, C-2′, and C-3′ were not determined because no synthetic 3,4,2′,3′-tetrahydroxy ceramides have been reported for comparison of NMR and optical rotation values. In addition the stereochemistries of these isolated ceramides is not possible to be unambiguously confirmed without further chemical transformations. However this would require reasonably more material to be available [1,3,4]. On the basis of all the evidence at our disposal in the present instance, the structure of **2** is suggested to be 1,3,4,2′,3′-pentahydroxy-2-octadecanoyl-amino-tricos-18-ene.

References

[1] K. O. Eyong, K. Krohn, H. Hussain, G. N. Folefoc, A. E. Nkengfack, B. Schulz and Q. Hu (2005). Newbouldiaquinone and newbouldiamide: A new naphthoquinone-anthraquinone coupled pigment and a new ceramide from *Newbouldia laevis. Chem. Pharm. Bull.* **53**, 616-619.

[2] M. Y. Bouberte, K. Krohn, H. Hussain, E. Dongo, B. Schulz and Q. Hu (2006) Tithoniamarin and tithoniamide: A new isocoumarin dimer and a new ceramide from *Tithnonia diversifolia Nat. Prod. Lett.* **20**, 842-849.

[3] M. Y. Bouberte, K. Krohn, H. Hussain, E. Dongo, B. Schulz and Q. Hu (2006) Tithoniaquinone A and tithoniamide B: A new anthraquinone and a new ceramide from the leaves of *Tithnonia diversifolia. Z. Naturforsch. B,* **61B**, 78-82.

[4] R.S. Miemanang, K. Krohn, H. Hussain and E. Dongo (2006). Paullinoside A and auallinomide A: A new cerebroside and a new ceramide from leaves of *Pullinia pinnata Z. Naturforsch. B*, **61B**, 1123-1127.

[5] E. Dongoa, H. Hussain, S. F. Kouam and K. Krohn (2010). Pycnangloside: A new cerebroside from *Pycnanthus angolensis Nat. Prod. Commun.* **5**, 1795-1798.

[6] T.B. Valeriea, H. Hussain, G. Albertine, E. Dongo, I. Ahmed, M. Riaz and K. Krohn (2011). Citropremide and citropridone: A new ceramide and a new acridone alkaloid from Stem Bark of *Citropsis gabunensis. Hel. Chim. Acta*. **94**, 1035-1040.

[7] H. Hussain, A.T. Nyongha, E. Dongo, I. Ahmed and W. Zhang (2012). Melicilamide A: a new ceramide from Melecia sxcelsa. *Nat. Prod. Res*. (in print)

[8] S. Sugiyama, M. Honda, R. Higuchi and T. Komori (1991). Biologically active glycosides from asteroidea, XXVI. Stereochemistry of the four diastereomers of ceramide and ceramide lactoside. *Liebigs Ann*. 349-356.

[9] M. Inagakia, R. Isobeb, Y. Kawanoa, T. Miyamotoa, T. Komoria and R. Higuchi (1998). Isolation and Structure of Three New Ceramides from the Starfish *Acanthaster planci. Eur. J. Org. Chem.* 129-131.