New Epoxy-Substituted Nitrogenous Bisabolene-Type Sesquiterpenes from a Hainan Sponge *Axinyssa* sp.

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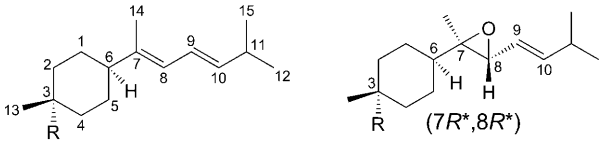
Two new uncommon epoxy-substituted nitrogenous bisabolene-type sesquiterpenes, 3-formamido-7,8-epoxy-α-bisabolane (**4**), 3-isocyano-7,8-epoxy-α-bisabolane (**5**), together with three known related sesquiterpenes, **1**–**3**, were isolated from the Hainan sponge *Axinyssa* sp. Their structures were determined on the basis of extensive spectroscopic analyses and by comparison of their NMR data with those of structurally related compounds.

Introduction. – Sponges of the genus *Axinyssa* have previously been reported to yield a variety of sesquiterpenes containing unusual nitrogenous functional groups, such as isothiocyanate, formamide, isonitrile, and thiocyanate [1–5]. Most of these N-containing compounds have exhibited various biological activities such as antithelmintic [1], antimicrobial [3], antimalarial [5] activities, and lethality for brine shrimp [6], which attracted considerable attention from natural-product chemists and pharmacologists.

As part of our ongoing research on the biologically active substances from Chinese marine invertebrates [7–10], we collected the sponge *Axinyssa* sp. off the Lingshui Bay, Hainan Province, P. R. China. Chemical investigation of the Et_2O-soluble fraction of an acetone extract of this sponge led to the isolation of two new uncommon epoxy-substituted nitrogenous bisabolene-type sesquiterpenes, 3-formamido-7,8-epoxy-α-bisabolane (**4**) and 3-isocyano-7,8-epoxy-α-bisabolane (**5**), together with three known related sesquiterpenes, 3-formamidotheonellin (**1**) [11–13], 3-isocyanotheonellin (**2**) [14], and 3-isothiocyanatotheonellin (**3**) [12] (*Fig. 1*). This report deals with the isolation and structure elucidation of the two new compounds.

Results and Discussion. – Freshly collected animals were immediately cooled to $-20°$ and kept frozen until used. Frozen material was extracted exhaustively with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble extract was subjected to repeated silica-gel and *Sephadex LH-20* column chromatography to afford two new sesquiterpenes, **4** and **5**, together with three known related sesquiterpenes, **1**–**3**.

The known sesquiterpenes were readily identified as 3-formamidotheonellin (**1**) [11–13], 3-isocyanotheonellin (**2**) [14], and 3-isothiocyanatotheonellin (**3**) [12], by comparison of their spectral data with those reported in the literature.



1 R = NHCHO
2 R = NC
3 R = NCS

4 R = NHCHO
5 R = NC

Fig. 1. *Structures of compounds* **1**–**5**

Compound **4** was isolated as a colorless oil. Its molecular formula was determined as $C_{16}H_{27}NO_2$ on the basis of HR-EI-MS (*m/z* 265.2043 (M^+; calc. 265.2041)), indicating four degrees of unsaturation. The ^{13}C-NMR and DEPT spectra of **4** indicated the presence of four Me groups (Me(12) (δ(C) 22.7), Me(13) (δ(C) 22.6 and 24.6), Me(14) (δ(C) 14.0), and Me(15) (δ(C) 22.7)), four sp^3-CH_2 groups (CH_2(1) (δ(C) 23.7 and 24.6), CH_2(2) (δ(C) 36.6 and 39.0), and CH_2(4) (δ(C) 36.6 and 39.0), and CH_2(5) (δ(C) 23.7 and 24.6)), three sp^3-CH groups (CH(6) (δ(C) 45.2 and 45.5), CH(8) (δ(C) 63.0), and CH(11) (δ(C) 31.1)), three sp^2-CH groups (CH(9) (δ(C) 121.7), CH(10) (δ(C) 144.6), and CHO (δ(C) 160.3 and 162.4)), and two sp^3-C-atoms (C(3) (δ(C) 52.6 and 53.8) and C(7) (δ(C) 64.4 and 64.7)), implying, from the required degrees of unsaturation, a monocyclic sesquiterpene framework. The ^{1}H-NMR spectrum of **4** provided evidences for four Me groups (δ(H) 1.35 and 1.42 (2*s*, each 1.5 H, Me(13)), 0.99 (*d*, J = 6.9, Me(12)), 0.99 (*d*, J = 6.9, Me(15)), and 1.20 (*s*, Me(14))), two olefinic H-atoms (δ(H) 5.28 (*dd*, J = 15.6, 7.3, H–C(9)) and 5.88 (*dd*, J = 15.6, 6.6, H–C(10))), and one trisubstituted epoxide group (δ(H) 3.17 (*d*, J = 7.5, H–C(8))). The NMR data of **4** (*Table*) were strongly reminiscent of those of the co-occurring sesquiterpene, 3-formamidotheonellin (**1**). In particular, the presence of the same formamide (NHCHO) functional group in **4** as in **1** is apparent. In fact, like in compound **1**, a characteristic doubling of the most ^{13}C-NMR signals due to the occurrence of rotational isomers of the formamide group were observed, indicating that **4** is also a formamide-bearing sesquiterpenes. A comparison of the overall ^{1}H- and ^{13}C-NMR data (*Table*) revealed that main differences between **4** and **1** are at C(7) and C(8) (C=C bond in **1**, epoxy group in **4**), in agreement with the observed molecular weight differences of 16 mass units. The presence of the epoxy group at C(7) and C(8) was further supported by distinct ^{1}H,^{1}H-COSY correlations between H–C(8) (δ(H) 3.17 (*d*, J = 7.5)) and H–C(9) (δ(H) 5.28 (*dd*, J = 15.6, 7.3)), H–C(9) and H–C(10) (δ(H) 5.88 (*dd*, J = 15.6, 6.6)), H–C(10) and H–C(11) (δ(H) 5.28 (*m*)), and H–C(11) and Me(12) (δ(H) 0.99 (*d*, J = 6.9)) and Me(15) (δ(H) 0.99 (*d*, J = 6.9)), and strong HMBC correlations between Me(14) (δ(H) 1.20, *s*) and C(7) (δ(C) 64.4, 64.7), C(6) (δ(C) 45.2, 45.5), and C(8) (δ(C) 63.0), between H–C(8) and C(6), C(9) (δ(C) 121.7), and C(10) (δ(C) 144.6) (*Fig. 2*). These spectroscopic evidences indicated that compound **4** is an α-bisabolene-type sesquiterpene with an 7,8-epoxy ring.

There are two stereogenic centers (C(7) and C(8)) in **4**. The configuration at C(3) and C(6) of **4** is proposed to be the same as in **1** *trans*, because, in the two compounds, the ^{13}C-NMR chemical shifts of the sp^3-C-atoms of the six-membered ring including

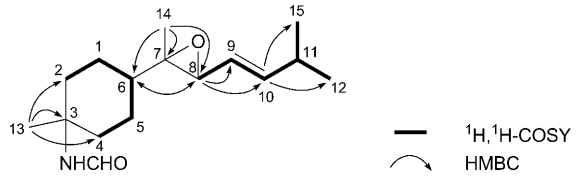


Fig. 2. *Selected ^{1}H,^{1}H-COSY correlations and HMBCs for compound* **4**

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *for Compounds* **4** *and* **5**[a]). δ in ppm, J in Hz.

	4[b])		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.37–1.41 (*m*, H_β), 1.63–1.67 (*m*, H_α)	23.7, 24.6 (2*t*)	1.28–1.32 (*m*, H_β), 1.70–1.75 (*m*, H_α)	24.5 (*t*)
CH_2(2)	1.58–1.62, 1.68–1.72 (2*m*, each 0.5 H, H_β), 1.83–1.87, 2.05–2.10 (2*m*, each 0.5 H, H_α)	36.6, 39.0 (2*t*)	1.70–1.74, 1.76–1.80 (2*m*, each 0.5 H, H_β), 1.76–1.82, 1.98–2.02 (2*m*, each 0.5 H, H_α)	38.2 (*t*)
C(3)	–	52.6, 53.8 (2*s*)	–	56.5 (*s*)
CH_2(4)	1.58–1.62, 1.68–1.72 (2*m*, each 0.5 H, H_β), 1.83–1.87, 2.05–2.10 (2*m*, each 0.5 H, H_α)	36.6, 39.0 (2*t*)	1.70–1.74, 1.76–1.80 (2*m*, each 0.5 H, H_β), 1.76–1.82, 1.98–2.02 (2*m*, each 0.5 H, H_α)	38.2 (*t*)
CH_2(5)	1.36–1.42 (*m*, H_β), 1.63–1.67 (*m*, H_α)	23.7, 24.6 (2*t*)	1.27–1.33 (*m*, H_β), 1.70–1.75 (*m*, H_α)	24.5 (2*t*)
H–C(6)	1.97–2.16 (*m*)	45.2, 45.5 (2*d*)	1.96–2.03 (*m*)	44.2 (*d*)
C(7)	–	64.4, 64.7 (2*s*)	–	64.3 (*s*)
H–C(8)	3.17 (*d*, J = 7.5)	63.0 (*d*)	3.17 (*d*, J = 7.2)	62.7 (*d*)
H–C(9)	5.28 (*dd*, J = 15.6, 7.3)	121.7 (*d*)	5.28 (*dd*, J = 12.6, 7.2)	121.6 (*d*)
H–C(10)	5.88 (*dd*, J = 15.6, 6.6)	144.6 (*d*)	5.86 (*dd*, J = 12.6, 6.6)	144.8 (*d*)
H–C(11)	2.30–2.40 (*m*)	31.1 (*d*)	2.35–2.42 (*m*)	31.1 (*d*)
Me(12)	0.99 (*d*, J = 6.9)	22.7 (*q*)	0.99 (*d*, J = 6.9)	22.1 (*q*)
Me(13)	1.35, 1.42 (2*s*, each 1.5 H)	22.6, 24.6 (2*q*)	1.44 (*s*)	24.6 (*q*)
Me(14)	1.20 (*s*)	14.0 (*q*)	1.19 (*s*)	14.2 (*q*)
Me(15)	0.99 (*d*, J = 6.9)	22.7 (*q*)	0.99 (*d*, J = 6.9)	22.1 (*q*)
NH	5.20 (br. *s*, 0.5 H), 5.89 (*d*, J = 9.3, 0.5 H)			
CHO	8.02 (*d*, J = 1.8, 0.5 H), 8.30 (*d*, J = 12.6, 0.5 H)	160.3, 162.4 (2*d*)		–
NC		–		152.3 (*s*)

[a]) δ Values referenced to $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard. Assignments deduced from the analysis of mononuclear and heteronuclear spectra. [b]) Most ^{1}H- and ^{13}C-NMR signals for **4** were doubled due to the presence of a formamide group.

that of Me(13) [11][12] are almost the same (δ(C) 22.6 and 24.6 for **4** *vs.* 22.1, 24.3 for **1**), as are the shapes of the ^{1}H-NMR signals for H–C(6). The characteristic upfield ^{13}C-NMR chemical shift of C(14) (δ(C) < 20 ppm) implied the (7*R**,8*R**)-configuration for the epoxy ring [15]. Although the relative configurations of C(7) and C(8) were elaborated as mentioned above, the relative configurational relationship between

C(6) and C(7) could not be determined by NOESY experiment due to the rotation of the C(6)–C(7) bond. Finally, the (*E*)-configuration of the C(9)=C(10), bond was determined by a 15.6-Hz coupling constant between H–C(9) and H–C(10). On the basis of the above evidences, the structure of **4** was determined as *trans*-3-formamido-7,8-epoxy-α-bisabolane.

Compound **5** was also isolated as a colorless oil. Its molecular formula, $C_{16}H_{25}NO$, was established by HR-EI-MS (*m/z* 247.1948 (M^+; calc. 247.1936)), 18 mass units less than that of **4**. The IR, and ^{1}H- and ^{13}C-NMR spectra of **5** closely resembled those of **4**. In fact, careful comparison of the NMR data (*Table*) of **5** with those of **4** revealed that the only difference between **5** and **4** is the substituent at C(3), where the NHCHO group in **4** was replaced by the NC group in **5**. Further, the presence of the $-N\equiv C$ group in **5** was confirmed by a diagnostic fragment ion peak at *m/z* 221 due to the loss of $-N\equiv C$ group from the molecular ion peak (*m/z* 247) in the EI-MS spectrum, and the ^{13}C-NMR signals at δ(C) 152.3 (NC) and 56.5 C(3). Detailed analysis of the 2D-NMR spectra allowed the unambiguous elucidation of the structure of **5**. The relative configuration at C(3), C(6), C(7), and C(8) were tentatively assigned the same as those in **4** based on a biogenetic consideration. Thus, the structure of compound **5** was elucidated as *trans*-3-isocyano-7,8-epoxy-α-bisabolane.

Nitrogenous bisabolene-type sesquiterpenes are frequently encountered in marine invertebrates [16]. However, epoxy-bearing nitrogenous bisabolene-type sesquiterpenes are relatively rare. To the best of our knowledge, this is the first report on the isolation of 7,8-epoxy-bisabolene sesquiterpenes from a natural source. It is apparent that **4** and **5** are formally derived from the co-occurring 3-formamidotheonellin (**1**) and 3-isocyanotheonellin (**2**), respectively, by the oxidation at the C(7)=C(8) bond.

It is interesting to note that a series of N-containing sesquiterpenes, which are structurally related to compounds **1**–**5**, were isolated from Hainan nudibranches *Hexabranchus sangueneus* [17] and *Phyllidiella pustulosa* [18] by our group. In particular, isolation of compound **2** from both the sponge *Axinyssa* sp. and the nudibranch *P. pustulosa* implied that the nudibranche might acquire the metabolites by dietary transfer from the title sponge.

Many bisabolene-type sesquiterpenes exhibited broad bioactivities such as antithelmintic [1], antimicrobial [3], antimalarial [5] activities, and lethality for brine shrimp [6]. Compounds **1**–**5** have been evaluated for cytotoxity against several tumor cell lines, but they were all inactive at a concentration of 10 mg/ml. Other bioassay studies such as anti-inflammatory and antimicrobial activities are currently underway.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; 200–300 mesh; *Qing Dao Hai Yang Chemical Group Co.*), and *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60*, *F-254*). Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *NicoletMagna FT-IR 750* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian Mercury 400* (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer; chemical shifts δ in ppm, with

residual $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard, coupling constant *J* in Hz. ^{1}H- and ^{13}C-NMR assignments were supported by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. EI-MS and HR-EI-MS: *Finnigan-MAT-95* mass spectrometer; in *m*/*z*.

Biological Material. Specimens of *Axinyssa* sp., identified by *R. v. S.* of the Zoological Museum, University of Amsterdam, were collected off Lingshui Bay, Hainan Province, P. R. China, in 2001, at a depth of -10 m, and were frozen immediately after collection. A voucher specimen (No. LS-340) is available for inspection with the Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The frozen animals (25 g dry weight) were cut into small pieces and exhaustively extracted with acetone ($0.8\,l \times 3$) at r.t. The extract was concentrated, and the resulting residue was partitioned between Et_2O and H_2O. The Et_2O-soluble portion was fractionated by CC (SiO_2; light petroleum ether (PE) with increasing amounts of acetone): eight fractions were obtained. *Fr. 2* was further purified over SiO_2 column (PE/Et_2O) and *Sephadex LH-20* column ($CHCl_3$) to afford **1** (20.0 mg), **2** (3.0 mg), **3** (5.3 mg), **4** (5.4 mg), and **5** (5.0 mg), resp.

3-Formamido-7,8-epoxy-α-bisabolane (= N-*(*trans-*1-Methyl-4-{(2*R**,3*R**)-2-methyl-3-[(1*E*)-3-methylbut-1-en-1-yl]oxiran-2-yl}cyclohexyl)formamide*; **4**). Colorless oil. $[\alpha]_D^{26} = 2.0$ ($c = 0.25$, $CHCl_3$). IR (KBr): 3299, 3049, 2958, 2977, 2867, 1668, 1537, 1463, 1272, 1386, 970. ^{1}H- and ^{13}C-NMR: *Table*. HR-EI-MS: 265.2043 ($C_{16}H_{27}NO_2^+$, calc. 265.2041).

3-Isocyano-7,8-epoxy-α-bisabolane (= trans-*1-Methyl-4-{(2*R**,3*R**)-2-methyl-3-[(1*E*)-3-methylbut-1-en-1-yl]oxiran-2-yl}cyclohexyl Isocyanide*; **5**). Colorless oil. $[\alpha]_D^{26} = 0.0$ ($c = 0.25$, $CHCl_3$). IR (KBr): 3049, 2977, 2958, 2867, 2139, 1460, 1386, 970. ^{1}H- and ^{13}C-NMR: *Table*. HR-EI-MS: 247.1948 ($C_{16}H_{25}NO^+$, calc. 247.1936).

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