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# Shinsonefuran, a cytotoxic furanosesterterpene with a novel carbon skeleton, from the deep-sea sponge *Stoeba extensa*☆

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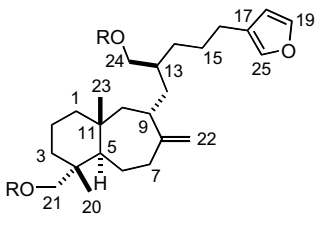
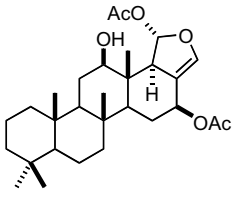
**Abstract**—Shinsonefuran, a cytotoxic sesterterpene with a novel carbon skeleton, was isolated from the deep-sea sponge *Stoeba extensa*. The gross structure and relative stereochemistry of shinsonefuran were determined by spectral and chemical methods. In addition, relative stereochemistry of halisulfate 7, previously reported from a marine sponge *Coscinoderma* sp., was revised. Shinsonefuran exhibited cytotoxicity against HeLa cells with an $IC_{50}$ value of 16 μg/mL.


Marine sponges inhabiting in deep seas have attracted the attention of marine natural products chemists because they are expected to have metabolic pathways different from those of shallow water species. In fact, some metabolites isolated from deep-sea species are structurally unique and biologically interesting as represented by discodermolide,[2a] halichondrin B,[2b] superstolides A[2c] and B,[2d] dercitin,[2e] and topsentin.[2f] In our search for anticancer leads from Japanese marine invertebrates, the marine sponge *Stoeba extensa*[3] collected in southern Japan showed significant cytotoxicity and bioassay-guided fractionation resulted in the isolation of a new compound shinsonefuran (**1**) together with the known sesterterpenes, 12β-hydroxyheteronemin (**2**) and halisulfate 7 (**3**). The present paper describes the isolation and structure elucidation of **1** and structural revision of **3**.

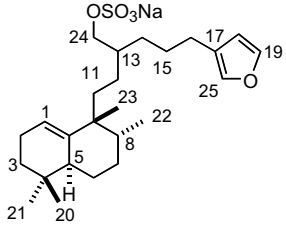
The frozen sample of *S. extensa* (160 g, wet weight) was extracted with MeOH (1 L × 3) whose extract was




**1 R = H**
**1a R = Ac**




**2**




**3**

☆ See Ref. 1.

*Keywords*: Deep-sea sponge; *Stoeba extensa*; Shinsonefuran; Sesterterpenes; Cytotoxic.

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partitioned between $H_2O$ and ether. The concentrated ether extract was partitioned between 90% MeOH and hexane and the aqueous MeOH layer was adjusted to 60% MeOH and extracted with $CHCl_3$. The active $CHCl_3$ fraction was separated on Sephadex LH-20 with MeOH to yield five fractions. Fraction 3 was further purified on silica gel ($CHCl_3$/MeOH, 95:5) followed by reversed-phase HPLC (MeOH/$H_2O$ 85:15) to furnish shinsonefuran (**1**, 4 mg) and 12β-hydroxyheteronemin (**2**, 50 mg). Fraction 4 was purified on silica gel with $CHCl_3$/MeOH (9:1) to yield halisulfate 7 (**3**, 25 mg).

Shinsonefuran (**1**) was obtained as a yellowish oil.[4] The molecular formula of $C_{25}H_{40}O_3$ was established by HRFABMS and $^{13}C$ NMR data, requiring six degrees of unsaturation. The FAB mass spectrum exhibited ion peaks at $m/z$ 389 $[M+H]^+$, 371 $[M-H_2O+H]^+$, and 353 $[M-2H_2O+H]^+$, indicating the presence of two hydroxyl groups, which was confirmed by the formation of a diacetate **1a**.[5] The $^1H$ NMR spectrum revealed signals ascribable to two singlet methyls (0.71 and 0.95 ppm), β-substituted furan (6.29, 7.25, and 7.37 ppm), a pair of exomethylene protons (4.56 and 4.75 ppm), two pairs of hydroxymethyls [2.97 (1H), 3.38 (1H), 3.39 (1H), and 3.42 (1H)] together with allylic and aliphatic protons (Table 1).

Although aliphatic regions of the $^1H$ NMR spectrum were severely overlapped between 1.2 and 1.6 ppm, carbon signals were well resolved. Therefore, we constructed the gross structure of **1** mainly on the basis of HMBC data. We started interpretation of HMBC data from the β-substituted furan: C-17 was correlated with $H_2$-15 ($\delta$ 1.52 and 1.60) and $H_2$-16 [$\delta$ 2.41 (2H)], the latter of which was further correlated with C-14 ($\delta$ 31.4). On the other hand, $H_2$-24 ($\delta$ 3.39 and 3.42) were correlated with two methylene carbons [C-12 ($\delta$ 40.0) and C-14 ($\delta$ 31.4)] and a methine carbon [C-13 ($\delta$ 38.2)], thus constructing the structure of the side chain.[6] A hydroxymethyl group ($\delta_H$ 3.39, 3.42; $\delta_C$ 65.9, C-24) could be attached at C-13 on the basis of HMBC cross-peaks, $H_2$-24/C-13 and C-12.

Similarly, the remaining bicyclic portion was constructed on the basis of HMBC data. The exomethylene protons ($H_2$-22) exhibited intense cross-peaks with C-7,

**Table 1.** NMR data[a] of shinsonefuran (**1**) in $CD_3OD$

| Position | $\delta_C$[b] | # | $\delta_H$ | Multiplet ($J$ in Hz) | HMBC (H to C) |
|---|---|---|---|---|---|
| 1 | 45.5 | α | 1.15 | dt (3.5, 13.5) | |
| | | β | 1.32 | m | |
| 2 | 19.5 | α | 1.40 | m | |
| | | β | 1.64 | Tq (3.0, 13.5) | |
| 3 | 37.6 | α | 1.49 | dt (4.2, 13.1) | C-2, 4, 20 |
| | | β | 1.19 | m | |
| 4 | 39.6 | | | | |
| 5 | 50.9 | | 1.30 | m | |
| 6 | 29.2 | α | 1.87 | tdd (2.4, 6.0, 13.2) | C-5, 7, 8 |
| | | β | 1.25 | m | |
| 7 | 34.6 | α | 2.00 | dt (2.4, 12.0) | C-5, 6, 8, 9, 22 |
| | | β | 2.23 | ddd (2.4, 6.0, 13.2) | C-5, 6, 8, 9, 22 |
| 8 | 157.4 | | | | |
| 9 | 40.3 | | 2.37 | m | C-8, 10, 12 |
| 10 | 53.9 | α | 1.22 | m | C-8, 9, 23 |
| | | β | 1.38 | m | |
| 11 | 37.8 | | | | |
| 12 | 40.0 | a | 1.09 | ddd (5.4, 7.8, 13.8) | C-8, 9, 10, 13, 14, 24 |
| | | b | 1.26 | m | C-8, 9, 10, 13, 14, 24 |
| 13 | 38.2 | | 1.57 | m | C-12, 14, 15 |
| 14 | 31.4 | | 1.35 | m | C-13, 15, 16 |
| 15 | 28.2 | a | 1.52 | m | C-13, 14, 16, 17 |
| | | b | 1.60 | m | C-14, 16, 17 |
| 16 | 26.0 | | 2.41 | dt (3.6, 7.2) | C-14, 15, 17, 25 |
| 17 | 126.4 | | | | |
| 18 | 111.9 | | 6.29 | dd (0.6, 1.8) | C-17, 19, 25 |
| 19 | 143.9 | | 7.37 | t (1.8) | C-17, 18, 25 |
| 20 | 18.2 | | 0.71 | s | C-3, 4, 5, 21 |
| 21 | 72.0 | a | 2.97 | d (11.4) | C-3, 4, 5, 20 |
| | | b | 3.38 | d (11.4) | C-3, 4, 5, 20 |
| 22 | 110.5 | a | 4.56 | d (2.4) | C-6, 7, 8, 9 |
| | | b | 4.75 | d (2.4) | C-6, 7, 8, 9 |
| 23 | 19.2 | | 0.95 | s | C-1, 5, 10, 11 |
| 24 | 65.9 | a | 3.39 | dd (6.2, 10.8) | C-12, 13, 14 |
| | | b | 3.42 | dd (5.4, 10.8) | C-12, 13, 14 |
| 25 | 140.1 | | 7.25 | td (1.2, 2.4) | C-17, 19 |

[a] Measured at 600 MHz ($^1H$) and 150 MHz ($^{13}C$).
[b] Multiplicities were determined by a DEPT 135 spectrum.

C-8, and C-9; $H_2$-7 ($\delta$ 2.00 and 2.23) were correlated with C-5 ($\delta$ 50.9) and C-6 (29.2); and H-9 ($\delta$ 2.37) was correlated with C-10 ($\delta$ 53.9). A singlet methyl ($\delta$ 0.71; $CH_3$-20) and the second hydroxymethyl ($\delta$ 2.97 and 3.38; $H_2$-21) could be placed on the same quaternary carbon (C-4) based on HMBC correlations from both $CH_3$-20 and $H_2$-21 to C-3, C-4, and C-5. Starting from the C-3 methylene protons, interpretation of the COSY spectrum led to three contiguous methylenes (C-1 to C-3). Therefore, the carbon framework from C-1 to C-10 was established. The remaining singlet methyl ($\delta$ 0.95; $CH_3$-23) was correlated with C-1, C-5, C-10 ($\delta$ 53.9), and C-11 (37.8), thus completing a bicyclo[5.4.0]undecane skeleton. The side chain was attached at C-9 as shown by the HMBC cross-peaks from H-12 ($\delta$ 1.09) to C-8, C-9, and C-10.

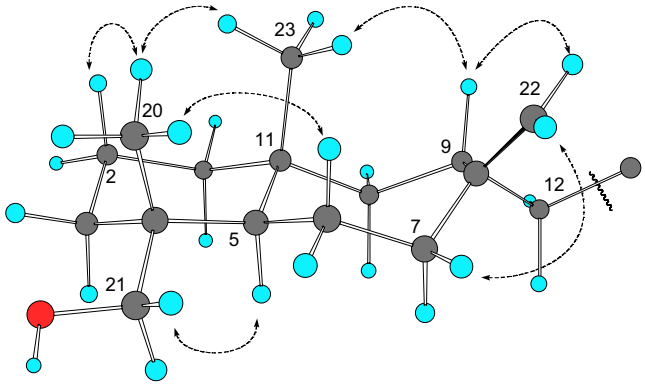


**Figure 1.** Selected NOESY correlations of shinsonefuran (**1**).

Intense NOESY cross-peaks among $CH_3$-20, $CH_3$-23, and H-2b suggested that their axial orientation on the six-membered ring, which was in the chair form as well as the *trans*-ring junction of the bicyclic system (Fig. 1). Similarly, NOESY cross-peaks between $CH_3$-23 and H-9, and between H-9 and H-22a indicated that they were on the same face of the seven-membered ring. To determine the relative stereochemistry at C-13, **1** was converted to a tricyclic ethers: treatment of **1** with *p*-TsOH in the presence of silica gel afforded an inseparable 2:1 mixture of **4** and **5**, which were epimeric at C-8.[7] Coupling constants between $H_2$-24 and H-13 showed that H-13 was equatorial in **4** and axial in **5**. Although interpretation of the NOESY data was hampered by severely overlapped signals in the aliphatic region, $^{13}C$ shifts of the newly generated singlet methyls ($\delta$ 26.2 in **4** and 18.2 in **5**) suggested that the tetrahydropyranyl ring was *cis*-fused in **4** and *trans*-fused in **5**.[8] NOESY cross-peaks between $CH_3$-22 and the axial proton on C-24 observed in both **4** and **5** indicated that the tetrahydropyran ring adopted a chair conformation. From these data, the relative stereochemistry of C-13 was assigned as shown.

Compound **3** was readily identified as halisulfate 7, a sesterpene sulfate isolated from a Micronesian sponge *Coscinoderma* sp., on the basis of the NMR data.[9] Although the carbon chemical shifts for the bicyclic portion of **3** were nearly identical ($\Delta\delta$ 0.0–0.5 ppm) to those of mycaperoxide H[10] and halisulfates 9 and 10,[11] an opposite stereochemistry was assigned at C-8. The stereochemistry of halisulfate 7 was proposed to be identical with that of *epi*-agelasine C[12] by comparing their $^{13}C$ NMR data. As pointed out previously,[10,13] there is an apparent confusion in the assignment of the relative stereochemistries in the bicyclic portion of the halimane skeleton. However, careful examination of NOESY data, which demonstrated cross-peaks (H-1/H-2a, H-2b/$CH_3$-21, H-5/H-11a, H-6a/H-8, and H-1/$CH_3$-23), led to the revision of stereochemistry of C-8 in halisulfate 7.

Shinsonefuran possesses a novel sesterterpene skeleton related to that of wriddol, a sesquiterpene isolated from the plant *Widdringtonia juniperoides*[14] Shinsonefuran (**1**), 12β-hydroxyheteronemin (**2**), and halisulfate 7 (**3**) were cytotoxic against HeLa cells with $IC_{50}$ values of 16, 0.7, and 16 μg/mL, respectively.

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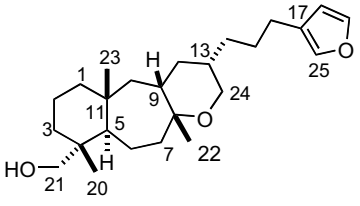
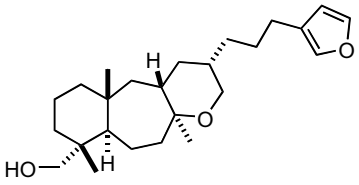



**4**




**5**

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3. The sponge was collected at Oshima-shinsone (28°52′N, 129°33′E) by dredging (ca 160 m deep) and identified as *Stoeba extensa* Dendy, 1905 (Demospongiae, Astrophorida, Pachastrellidae): Sponge forming a thin membrane agglutinating reef debris and sand, color dark black-purple. The ectosomal skeleton consists of a dense cover of microscleres. The choanosomal skeleton is a closely arranged mass of megascleres arranged without apparent order. Megascleres consist of thick shafted calthrops-like triaenes and dichotriaenes, rhabdome 120–200 × 12–25 μm, cladi somewhat shorter. Dichotriaenes less abundant than calthropses. Microscleres are sanidasters of uniform size and shape, densely spined, 18–25 × 3 μm. The sponge conforms closely to the description of the type from Sri Lanka. The voucher was deposited at Institute for Systematics and Ecology, University of Amsterdam as ZMA Por. 17646.
4. Shinsonefuran (**1**): yellow oil, $[\alpha]_D^{21}$ +39.0° (*c* 0.20, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 211 (3.77); $^1$H and $^{13}$C data in $CD_3OD$ (see Table 1); HRFABMS *m*/*z* 389.3055 [(M+H)$^+$, calcd for $C_{25}H_{41}O_3$, 389.3056].
5. Shisonefuran diacetate (**1a**): pale yellow oil; $^1$H NMR ($CD_3OD$, 600 MHz) δ 7.38 (1H, br s, H-20), 7.26 (1H, br s, H-19), 6.29 (1H, br s, H-21), 4.77 and 4.57 (1H each, d, *J* = 2.4 Hz, H-9), 3.98 (2H, m, H-25), 3.92 (1H, d, *J* = 11.2 Hz, H-23), 3.62 (1H, d, *J* = 11.5 Hz, H-23), 2.51 (2H, q, *J* = 7.3 Hz, H-17), 2.26 (1H, m, H-7b), 2.04 (3H, s, OCO*CH*$_3$), 2.02 (1H, m, H-7a), 1.99 (3H, s, OCO*CH*$_3$), 1.95 (1H, m, H-6b), 0.97 (3H, s, $CH_3$-24), 0.82 (3H, s, $CH_3$-22).
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