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SESQUITERPENE FURANS AND THIOSESQUITERPENES FROM THE NUDIBRANCH *CERATOSOMA BREVICAUDATUM*

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ABSTRACT.—Six furanosesquiterpenes have been isolated from an Australian nudibranch, *Ceratosoma brevicaudatum*, and their structures were determined by spectral analysis. The metabolites include the known terpenes dehydrodendrolasin [**1**], dehydrolasiosperman [**3**], and thiofurodysinin acetate [**7**]. The remaining metabolites were determined to be an unreported *cis* isomer **2** of dehydrodendrolasin, (methylthio) furodysinin [**4**], and dithiofurodysinin disulfide [**5**], derivatives of thiofurodysinin acetate that had been isolated earlier from a sponge. ^{13}C-nmr data were obtained for all compounds.

One of the defense strategies for nudibranchs involves the sequestering of metabolites from their sponge diet as chemical defense agents (1–3). Included among the reported assimilated defensive chemicals are a variety of terpene metabolites. Recently, we reported the isolation of a group of spongiane diterpenes from several nudibranchs [tentatively identified then as *Ceratosoma brevicaudatum* (Abraham, 1876; Chromodorididae), but now corrected to *Chromodoris epicuria* (Basedow and Hedley)] that were collected near Adelaide, South Australia (4). Subsequently, we collected another batch of nudibranchs from the same area and these have been identified (after extraction of the combined batch) as a mixed collection of *Ch. epicuria* and *Ce. brevicaudatum* with the latter predominating. Juvenile *Ce. brevicaudatum* look very similar to *Ch. epicuria* (and to several other species) in the field, and, consequently, upon superficial examination at the time of collection all specimens were thought to be of the same species. From this mixed collection of nudibranchs we have isolated three known furanosesquiterpenes, dehydrodendrolasin [**1**] (5), dehydrolasiosperman [**3**] (6,7), thiofurodysinin acetate [**7**] (8–10), and three new furanosesquiterpenes, **2**, **4**, and **5**. The very uncommon sulfur-containing sesquiterpene **7** was isolated first from an unidentified species of sponge, *Dysidea* sp. (8,9), and again recently from *Dysidea avara* where it was found together with the free thiol **8** (10). These compounds and the related thiofurodysin acetate appear to be the only naturally occurring terpene thiol derivatives reported to date (8).

RESULTS AND DISCUSSION

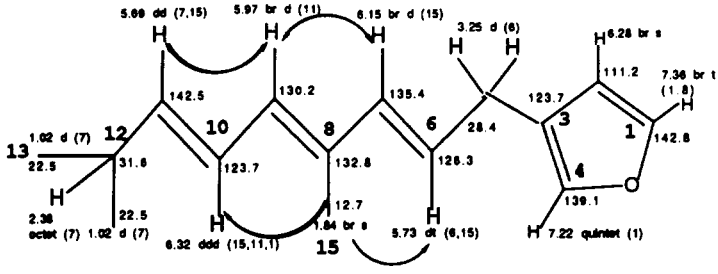
Taxonomic analysis of the specimens was performed after extraction by examination of the jaw plates, and this revealed that the collection of nudibranchs consisted of a large, medium, and small sized specimen of *Ce. brevicaudatum* and one small specimen of *Ch. epicuria*. Thus, the chemistry of this mixed collection is assumed to derive from *Ce. brevicaudatum*.

The structure of dehydrodendrolasin was established from the nmr data shown around structure **1**. These data were derived from detailed analysis of ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, ^{1}H decoupling, and difference double resonance (DDR) nmr spectra which were measured in $CDCl_3$. The ^{1}H-nmr data are very similar to those obtained earlier (5) for **1** in CCl_4 including the coupling constants which confirmed the 6*E*, 10*E* configurations. The low field nmr chemical shift for C-15 (10) and the nOe's shown on structure **1** conclusively established the *E* configuration for the 8,9 double bond also. This latter aspect of the stereochemistry had not been resolved in the initial description of **1**.

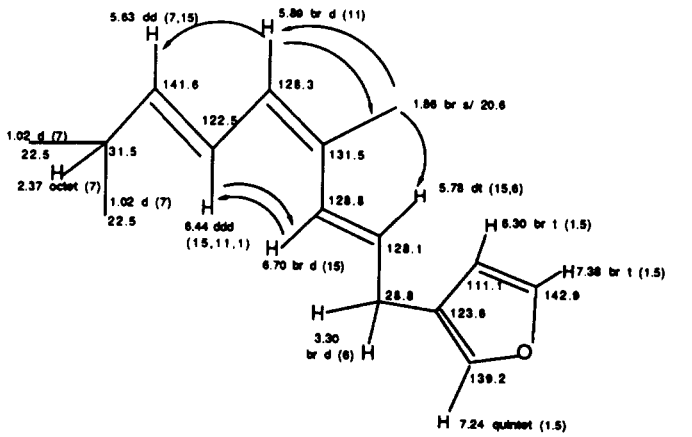
The formula of **2**, $C_{15}H_{20}O$, was established by low resolution ms analysis ($[M]^+$ 216) and ^{13}C-nmr data. The single oxygen in the formula was inferred from ^{1}H- and

^{13}C-nmr signals typical of a β-substituted furan ring. The structure of the entire side chain was verified by ^{1}H-nmr decoupling, DDR, and COSY experiments; see data on structure **2**. A ^{1}H-^{13}C COSY experiment confirmed the chemical shift assignments for the protonated carbons. An allylic coupling of 1.5 Hz was observed between the methylene proton signal at 3.30 ppm (H-5) and the furan α-proton at 7.24 ppm (H-4), thus establishing the connection between the side chain and C-3 of the furan ring. The 6*E*,8*Z*,10*E* stereochemistry shown was assigned on the basis of (a) the nOe interactions shown on structure **2**, (b) the 15 Hz coupling constants determined for H-6,7, and H-10,11, and (c) the chemical shift of C-15 (11).

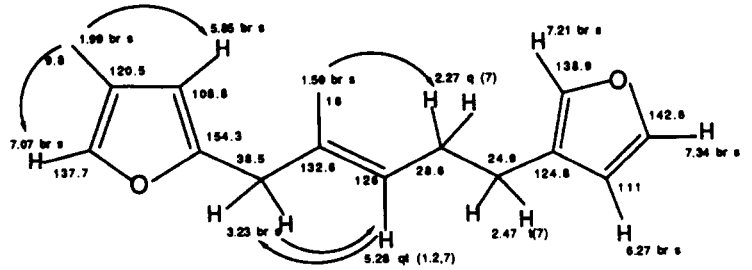
Hrms data confirmed the formula $C_{15}H_{18}O_2$ for sesquiterpene **3**. The structure was deduced from ^{13}C (DEPT) and ^{1}H-nmr data, including decoupling and nOe experiment results, and a COSY plot. The ^{1}H-nmr data obtained in $CDCl_3$ for this metabolite are very similar to those reported (6) for the plant metabolite dehydrolasiosperman [**3**] in CCl_4, and, hence, we conclude that these compounds are one and the same. ^{13}C-nmr data have not been reported previously for dehydrolasiosperman (7).

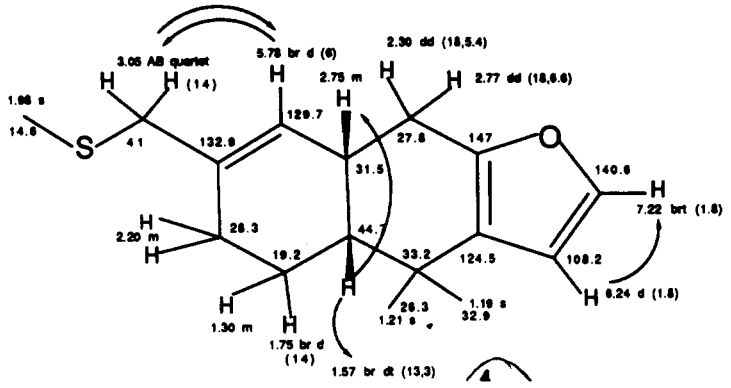


1

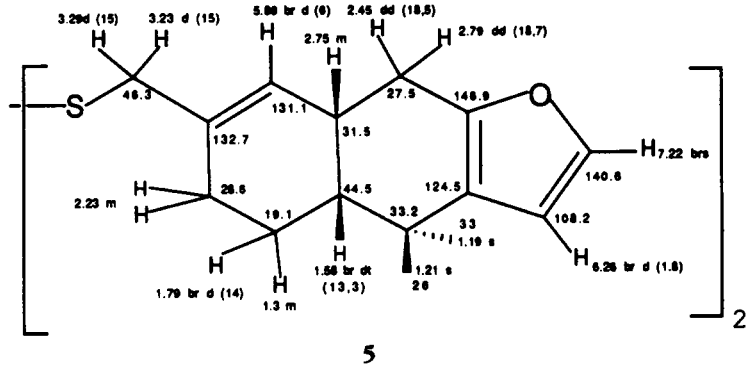


2

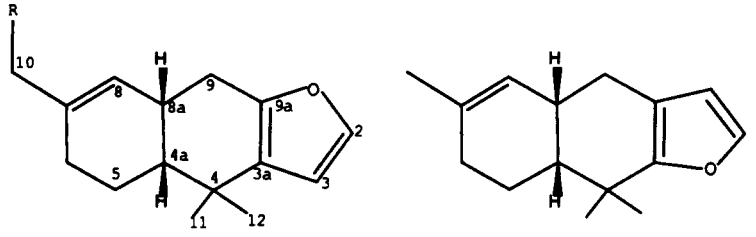




4



5



6 R=H
7 R=AcS
8 R=SH

9

The presence of one sulfur atom in the formula of **4**, $C_{16}H_{22}OS$, was suggested by a significant $[M+2]^+$ peak in the low resolution mass spectrum [12 eV, *m/z* 264 (4.8%), 262 (78.8%)], and this was confirmed by high accuracy mass measurement of the molecular ion (found 262.1389, calcd 262.1391). Sizeable $[M]^+$ and $[M+2]^+$ peaks were seen only in the 12 eV lrms; only a small $[M]^+$ ion was seen in the hrms. Hence, **4** has six degrees of unsaturation. The presence of a 2,3-disubstituted furan was inferred from the following carbon signals: 140.6 (d), 108.2 (d), 147.0 (s), and 124.5 (s) (12). $^1H/^1H$ COSY, ^1H decoupling, and DDR experiments, supplemented by $^1H/^{13}C$ COSY information, established all of the ^1H- and ^{13}C-nmr assignments for **4**. All the ^1H-^1H couplings needed to support proposed structure **4** were evident in the COSY spectra or were observed by decoupling, except that there were no ^1H-^1H couplings to provide a basis to connect the geminal dimethyl groups and their associated quaternary carbon to the remainder of the skeleton. This last aspect of the structure was resolved by

a selective INEPT (13) experiment (maximized for $J = 9$ Hz) in which the 1.57 ppm proton signal (H-4a) was irradiated. The resulting carbon spectrum showed signals for only C-4 (33.2), C-3a (124.5), and C-9 (27.8 ppm). This confirmed that the geminal dimethyl array must be incorporated in **4** as shown. A *cis* ring fusion was assigned to **4** based on nOe's observed between the ring juncture protons. The ^{13}C-nmr chemical shift of the methyl carbon of the thioether group is consistent with that of a model compound, methyl benzyl sulfide (14). Also, the ^{13}C-nmr chemical shifts of the furan carbons match those reported for furodysinin [**6**] and thiofurodysinin acetate [**7**] (8), and, hence, the furan ring is definitely oriented as shown in **4**. The ^{13}C-nmr shifts of the fully substituted furan carbons are quite different in the furodysin **9** (8) (113.0 and 156.8 ppm) which has an isomeric fusion of the furan ring. By analogy with the trivial name given to **7**, thioether **4** may be designated (methylthio) furodysinin.

The ^{1}H and ^{13}C spectra of the final compound isolated, **5**, were nearly identical to those of **4** except that the thiomethyl signal was missing in the former spectra, and slight differences in nmr chemical shifts were noted for the carbons and hydrogens at positions 7, 8, and 10 in **5** relative to those in **4**. Decoupling experiments confirmed that the two compounds have the same proton coupling interactions and, hence, have the same bicyclic ring structures. However, in the 12 eV low resolution mass spectrum of **5** the highest mass ion observed was at *m/z* 494, which indicated that the molecular weight of **5** was much greater than would be projected from the observation of only fifteen carbon signals in the ^{13}C-nmr spectrum. This prompted the proposal of a symmetrical disulfide structure **5**, $C_{30}H_{38}O_2S_2$, the molecular weight of which is consistent with the observed $[M]^+$ at 494 and the symmetry of which accounts for the occurrence of only 15 distinct signals in the ^{13}C-nmr spectrum. The disulfide functionality also nicely accounts for the ca. 5 ppm downfield shift of the ^{13}C-nmr signal of the thiomethylene carbon in **5** relative to that of the analogous carbon of **4**.[1] Metabolite **5**, dithiofurodysinin disulfide, may be an artifact arising from oxidative coupling of the corresponding thiol **8**, since thiols dimerize readily in air.

Systematic names for **4** and **5** are as follows: [**4**] 4,4-dimethyl-7-(methylthiomethyl)-*cis*-4,4a,5,6,8a,9-hexahydronaphtho[2,3-*b*]furan; [**5**] di-(4,4-dimethyl-*cis*-4,4a,5,6,8a,9-hexahydronaphtho[2,3-*b*]furan-7-yl) methyl disulfide.

Metabolites **4** and **5** are additions to the small group of sulfur-containing sesquiterpenes isolated previously from two sponges of the genus *Dysidea*. Their occurrence in the nudibranch *Ce. brevicaudatum* suggests that this mollusk feeds on *Dysidea* sponges or perhaps blue-green algae associated with the sponge.

EXPERIMENTAL

GENERAL EXPERIMENTAL CONDITIONS.—Experimental conditions used have been previously described (4). ^{1}H-nmr data shown on structures **1–5** were obtained in $CDCl_3$. High resolution mass spectra were obtained on a VG ZAB-E mass spectrometer.

SPECIMEN COLLECTION.—Specimens were collected around the jetty at Rapid Bay, South Australia at depths of approximately 15–25 feet and were preserved by freezing. After extraction the dried specimens and color photos taken at the collection sites were used for identification. Specimen remains and two alcohol-preserved specimens have been retained by Dr. Rudman.

ISOLATION AND PURIFICATION OF COMPOUNDS **1–5**.—The frozen nudibranchs (7 animals) were allowed to soak twice in Me_2CO (400-ml portions) for 3 h and 12 h successively, and finally in $CHCl_3$-MeOH (1:1) (400 ml) for 12 h. The resulting solvents were combined and evaporated to give 2 g of oily organic extract. The $CHCl_3$-soluble portion (trituration) of the combined extracts (1.4 g) was chromato-

[1]Cf. chemical shifts for the methylene carbons of ethyl sulfide, 25.6, and diethyl disulfide, 33.0 ppm, Sadtler Standard Carbon-13 Spectra, Sadtler Research Laboratories, Philadelphia, PA, 1976, Vol. 1, no. 74C, and Vol. 3, no. 419C, respectively.

graphed over a Si gel column using the following step-gradient elution (20-ml fractions): hexane (5 fractions), hexane-$CHCl_3$ (1:1) (3 fractions), and finally $CHCl_3$-MeOH (95:5) (6 fractions). Fractions 2 and 3 were combined and purified by hplc using two semi-preparative Si gel columns (25 cm and 30 cm, connected in series) and eluting with hexane to give compounds **1** (25 mg) and **2** (20 mg) as colorless oils. Fraction 7 was rechromatographed on a preparative tlc plate to give 3 fractions, a–c. Fractions a and b were purified further by hplc using a Si gel column and eluting with hexane to give compounds **3** (2.0 mg) and **4** (3.0 mg). Fraction c was purified by hplc using a C_{18} column and iPrOH as eluent to give compound **5** (1.5 mg). Fraction 8 was purified by hplc using a C_{18} column and iPrOH as eluent to give compound **7** (0.8 mg). Compounds **3**–**5** and **7** were also obtained as oils.

Mass spectral data for compounds **2**, **4**, *and* **5**.—*Compound* **2**.—Eims (70 eV) *m/z* (rel. int.) $[M]^+$ 216 (54), 173 (20), 135 (27), 105 (50), 81 (100).

Compound **4**.—Eims (12 eV) *m/z* (rel. int.) $[M+2]^+$ 264 (5), $[M]^+$ 262 (78), 215 (19), 122 (100).

Compound **5**.—Eims (12 eV) *m/z* (rel. int.) $[M]^+$ 494 (15), 279 (16), 256 (60), 236 (34), 215 (100).

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LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **3**, 1 (1986).
2. G. Cimino, S. de Rosa, S. de Stefano, R. Morrone, and G. Sodano, *Tetradedron*, **41**, 1093 (1985).
3. K. Gustafson and R.J. Andersen, *Tetrahedron*, **41**, 1101 (1985).
4. M.B. Ksebati and F.J. Schmitz, *J. Org. Chem.*, **52**, 3766 (1987).
5. G. Cimino, S. de Stefano, and E. Trivellone, *Tetrahedron*, **28**, 4761 (1972).
6. H. Bornowski, *Tetrahedron*, **27**, 4101 (1971).
7. F. Bohlmann and C. Zdero, *Phytochemistry*, **21**, 1989 (1982), and references cited therein.
8. R. Kazlauskas, P.T. Murphy, R.J. Wells, J.J. Daly, and P. Schonholzer, *Tetrahedron Lett.*, 4951 (1978).
9. R.W. Dunlop, R. Kazlauskas, G. March, P.T. Murphy, and R.J. Wells, *Aust. J. Chem.*, **35**, 95 (1982).
10. R.J. Capon and J.K. McLeod, *J. Nat. Prod.*, **50**, 1136 (1987).
11. P. Crews and S. Naylor, *Prog. Chem. Org. Nat. Prod.*, **48**, 203 (1985).
12. B.F. Bowden, J.C. Coll, E.D. de Silva, M.S.L. de Costa, P.J. Djura, M. Mahendran, and D.M. Tapiolas, *Aust. J. Chem.*, **36**, 371 (1983).
13. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
14. "Sadtler Standard Carbon-13 Spectra," Sadtler Research Laboratories, Philadelphia, PA, 1976, Vol. 15, no. 2924.

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