Pergamon

Tetrahedron Letters, Vol. 35, No. 32, pp. 5793-5796, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01196-6

The First *Seco*-Asbestinin: a Novel Class of Diterpene from the Caribbean Gorgonian *Briareum Asbestinum* (Pallas).

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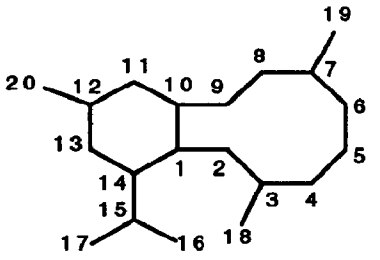
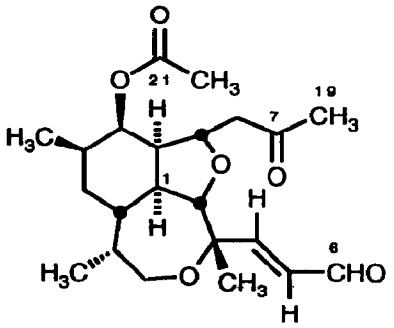
Abstract: The hexane extract of the Caribbean gorgonian octocoral *Briareum asbestinum* was found to contain traces of a novel *seco*-asbestinin diterpenoid. The structure of **1**, the first representative of this novel class of diterpenoids, was assigned on the basis of extensive NMR analyses and by comparison with analogous spectral data from related asbestinane diterpenoids. Confirmation of structure **1** was achieved by partial synthesis from known asbestinin-6 (**2**).

In our continuing survey of Puerto Rican marine invertebrates with promising cytotoxic activity against a variety of cancerous cell lines, we encountered the gorgonian *Briareum asbestinum* during a recent expedition to Mona Island off the West coast of Puerto Rico. This invertebrate has been the subject of intense investigations in our laboratory. We recently reported the isolation and structures of nine tetracyclic diterpenes representatives from the skeletal class asbestinanes.[1-3] Several of these compounds showed interesting pharmacological properties. In this communication we report the isolation, structure, and partial synthesis of compound **1**, which contains only three rings and thus comprises a novel type of ether-cyclized asbestinane diterpene.



asbestinane skeleton



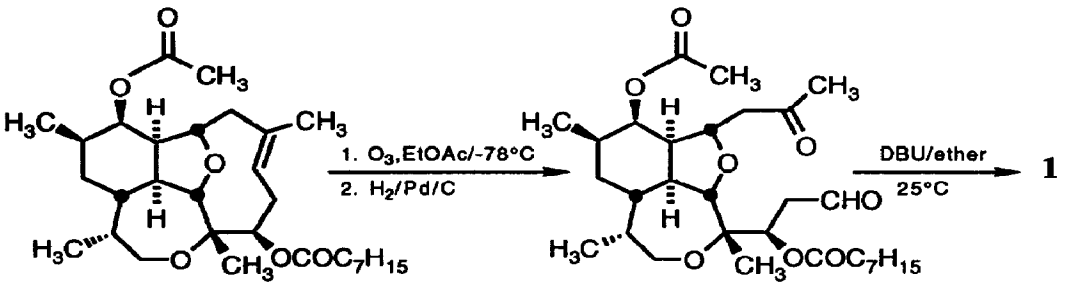
1

Compound **1** was isolated pure in trace amounts from the hexane extract of *B. asbestinum* after successive size exclusion (Bio-Beads SX-2 in toluene), silica gel column chromatography and reversed-phase HPLC (ODS-Si).[4] The high resolution

mass spectrum of the UV active metabolite [λ_{max} (MeOH) 214 nm (ε = 5,780)] established a molecular formula for this compound of $C_{22}H_{32}O_6$ (found *m/z* 392.22004 [M+], calcd 392.21996), which was supported by ^{1}H- and ^{13}C-NMR data. The HREIMS gave a strong mass peak at *m/z* 332.19880 for $C_{20}H_{28}O_4$, which reflects a fragmentation of [M-AcOH]$^+$ from the molecular ion. The ion peak at *m/z* 332 in turn fragments to another ion peak at *m/z* 274.15636 for $C_{17}H_{22}O_3$ representing the loss of one molecule of acetone. Absorptions in the IR indicated the presence of ester (1734 cm^{-1}), ketone (1718 cm^{-1}), and α,β-unsaturated (1692 cm^{-1}) carbonyl functionalities. The ^{1}H-nmr spectrum of compound **1** contained signals for one acetyl methyl at δ 2.12 (3H, s), two sp^2 methine protons [δ 6.83 (1H, d, J = 15.6 Hz) and 6.41 (1H, dd, J = 8.1, 15.6 Hz)], three oxymethine protons: one sp^2 [δ 9.57 (1H, d, J = 8.1 Hz)] and two sp^3 [δ 3.95 (1H, m) and 3.67 (1H, br d, J = 6.9 Hz)], two diastereotopic oxymethylene protons [δ 3.81 (1H, d, J = 12.9 Hz) and 3.62 (1H, dd, J = 3.0, 12.9 Hz)], one acetoxy proton signal [δ 5.18 (1H, br t)], and four methyl groups [δ 2.15 (3H, s), 1.43 (3H, s), 0.93 (3H, d, J = 6.9 Hz), and 0.92 (3H, d, J = 6.9 Hz)]. The ^{13}C-NMR spectrum showed the presence of twenty-two carbon atoms three of which are carbonyl signals at δ 206.22 (s), 194.07 (d) and 170.89 (s), two are olefinic carbons at δ 159.59 (d) and 130.71 (d), and five represent signals for carbon atoms bearing oxygen at δ 91.48 (d), 76.82 (s), 76.80 (d), 72.10 (d), and 68.18 (t). The ^{13}C-NMR resonances at δ 206.22 (s), 194.07 (d), 170.89 (s), 159.59 (d), and 130.71 (d) supported the presence of a ketone, an α,β-unsaturated aldehyde and ester groups. The ester was identified as an acetate by the presence of a methyl resonance in the ^{1}H-NMR spectrum at δ 2.12 (s, 3H) and from MS data. These three carbonyl groups and the carbon-carbon double bond accounted for four of the seven double-bond equivalents required by the molecular formula. Compound **1**, therefore, contained three rings in its structure. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of known asbestinin-6 (**2**)[2] confirmed many structural similarities among these compounds and also suggested the presence of some features unique to **1**. For instance, while the cyclohexane, tetrahydrofuran and oxepane ring moieties appeared to be intact in **1** on the basis of similar IR, MS, and NMR data, the remaining spectroscopic features indicated that in **1** the usual C-6, C-7 endocyclic double bond had been cleaved oxidatively to an aldehyde and ketone, respectively. For instance, unique to **1** was the aldehyde proton signal at δ 9.57 (d, 1H, J= 8.1 Hz) which was shown by COSY to be coupled only to the olefinic methine proton at δ 6.41 (1H, dd, J= 8.1, 15.6 Hz) which in turn was likewise shown by COSY to be coupled to the remaining olefinic proton at δ 6.83 (1H, d, J= 15.6 Hz). The large coupling (15.6 Hz) observed between H-4 (δ 6.83) and H-5 (δ 6.41) suggested a *trans* orientation for these two protons. This was supported by the absence of a NOE effect between these two signals. The presence of an α,β-unsaturated aldehyde functionality in **1** was also

supported by the UV absorption at λ_{max} 214 nm. The loss of acetone observed in the MS spectrum of **1** and the ^{1}H-NMR signal at δ 2.15 (s, 3H) combined with resonances in the ^{13}C-NMR spectrum at δ 206.22 (s) and 30.44 (q), indicated the presence in this compound of a methyl ketone moiety, a feature not found in any previously reported asbestinane diterpene. Compound **1**, with these unprecedented structural features, thus comprises a new group of ether-cyclized asbestinanes hereon known as *seco*-asbestinins.

The structure of **1** was confirmed through the following reaction sequence.[5] Ozonolysis of asbestinin-6 (**2**) in EtOAc solution at -78°C, followed by catalytic hydrogenation of the ozonide, gave the expected keto aldehyde **3**.[6] Treatment of the keto aldehyde at 25°C with DBU in ether caused β-elimination of caprylic acid, yielding compound **1**. As has been observed the asbestinin diterpenes possess potent biological activities in several pharmacological assays.[1] Compound **1**, however, showed no significant cytotoxic activity against the HeLa and CHO-K1 cell lines within concentration ranges of 5 to 250 μg/mL (*in vitro*).



2 **3**

Acknowledgments: The assistance of José J. Morales, Miriam Ortiz, Luis A. Ríos, Deborath Parrilla, Javier J. Soto and Dr. José E. García in specimen collection is gratefully acknowledged. We extend our sincere appreciation to the crew of the R/V Isla Magueyes for their assistance during a trip to Mona Island and to Dr. Fernando González from the Department of Chemistry at U.P.R. for performing the HeLa and CHO-K1 cytotoxicity tests. HREIMS spectral determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Facility (Grant No. CHE8211164). This study was supported in part by the National Science Foundation EPSCoR Program (Grant No. R118610677), the NIH-MBRS Program (Grant No. S06RR08102-17) and the NSF-MRCE Program (Grant No. R11-8802961). O. M. Cóbar thanks the University of San Carlos, Guatemala, and the Agency for International Development (AID) for a pre-doctoral fellowship.

References and Notes

1. Morales, J.J.; Lorenzo, D.; Rodríguez, A.D. *J. Nat. Prod.* **1991**, *54*, 1368.
2. Rodríguez, A.D.; Cóbar, O.M. *Tetrahedron* **1993**, *49*, 319.
3. Rodríguez, A.D. *Magn. Reson. Chem.* **1992**, *30*, 977.
4. Compound **1**: (colorless oil) $[\alpha]^{25}_{D}$ + 21.14° (*c* = 3.5, $CHCl_3$), UV λ_{max} (MeOH) nm (log ε) 214 (3.78); IR (neat) 2963, 2930, 2876, 1734, 1718, 1692, 1658, 1455, 1422, 1371, 1299, 1238, 1182, 1159, 1126, 1109, 1077, 1045, 1031, 1015, 989, 953, 939, 894, 875, 852, 830, 811, 801, 732, 686, 673, 622, 609, 592, 583 cm^{-1}; ^{1}H-NMR ($CDCl_3$, see text); ^{13}C-NMR ($CDCl_3$) δ 38.56 (d, C-1), 91.48 (d, C-2), 76.82 (s, C-3), 159.59 (d, C-4), 130.71 (d, C-5), 194.07 (d, C-6), 206.22 (s, C-7), 48.36 (t, C-8), 76.80 (d, C-9), 47.94 (d, C-10), 72.10 (d, C-11), 31.31 (d, C-12), 30.54 (t, C-13), 37.52 (d, C-14), 36.52 (d, C-15), 68.18 (t, C-16), 11.15 (q, C-17), 24.13 (q, C-18), 30.44 (q, C-19), 18.51 (q, C-20), 170.89 (s, C-21), 21.13 (q, C-22); HREIMS *m/z* $[M]^+$ 392.22004 (21.1%) ($C_{22}H_{32}O_6$ requires 392.21996), 332 (4.1), 274 (13.2), 251 (40.0), 233 (45.4), 191 (49.9), 175 (100), 147 (44.3), 133 (73.4), 105 (71.3), 93 (45.0), 55 (52.5).
5. A similar reaction sequence leading to a keto aldehyde such as **1** was used during a chemical degradation study of a related asbestinane diterpenoid; see Stierle, D.B.; Carté, B.; Faulkner, D.J.; Tagle, B.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 5088.
6. A stream of ozone in oxygen was bubbled into a solution of asbestinin-6 (**2**) (14.8 mg, 0.03 mmol) in ethyl acetate (10 mL) at -78°C until the solution turned blue. The solution was stirred for an additional 5 min and then warmed to room temperature while excess ozone was removed in a stream of dry nitrogen. Palladium on charcoal (10%) catalyst was added to the solution which was then stirred under an atmosphere of hydrogen for 4 hours. The catalyst was removed by filtration and the solvent evaporated to obtain the keto aldehyde **3** (9.3 mg, 59% yield): colorless oil, IR (neat) 2959, 2929, 2874, 1739, 1732, 1716, 1456, 1418, 1393, 1374, 1236, 1164, 1045, 1018, 802 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.87 (3H, t, J = 6.9 Hz), 0.93 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.9 Hz), 1.27 (3H, s), 2.11 (3H, s), 2.13 (3H, s), 2.28 (2H, dd, J = 2.4, 7.5 Hz), 2.60 (3H, m), 2.98 (1H, dd, J = 4.2, 16.2 Hz), 3.55 (1H, dd, J = 3.3, 13.2 Hz), 3.71 (1H, br d, J = 12.6 Hz), 3.87 (1H, m), 5.16 (1H, t, J = 3.3 Hz), 5.42 (1H, dd, J = 4.2, 7.2 Hz), 9.81 (1H, br t, J = 2.4 Hz); ^{13}C-NMR ($CDCl_3$) δ 206.59 (s), 202.69 (d)[7], 172.43 (s), 170.99 (s), 92.02 (d), 77.20 (d), 76.71(s), 72.05 (d), 71.12 (d), 68.38 (t), 48.16 (t), 47.86 (d), 38.86 (t), 38.03 (d), 36.81 (d), 36.71 (d), 34.51 (t), 31.65 (t), 31.48 (d), 30.72 (t), 30.66 (q), 29.05 (t), 28.97 (t), 24.97 (t), 22.61 (t), 21.17 (q), 20.68 (q), 18.71 (q), 14.06 (q), 10.98 (q); LREIMS $[M]^+$ 536 (23), 445 (20), 281 (38), 254 (19), 207 (57), 172 (26), 93 (50), 91 (63), 57 (100).
7. Because it was broad and of low intensity this resonance line may be inaccurate.

(*Received in USA* 25 *May* 1994; *accepted* 16 *June* 1994)