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Cephalotaxidine, a Novel Dimeric Alkaloid from *Cephalotaxus harringtonia* var. *drupacea*

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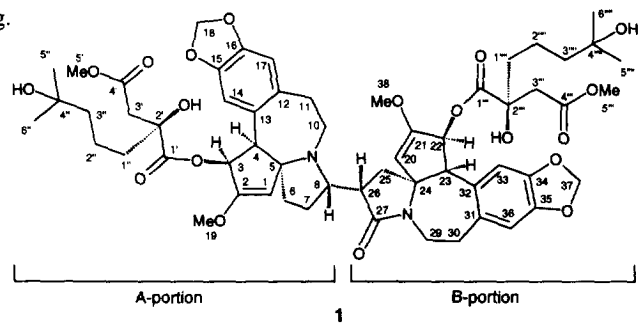
Abstract: Cephalotaxidine (**1**), a novel *Cephalotaxus* dimeric alkaloid, has been isolated from *Cephalotaxus harringtonia* var. *drupacea*, and its structure has been elucidated based on spectroscopic analyses. Alkaloid **1** has a unique C–C bridge between the two mother skeletons which are composed of homoharrigtonine and its oxygenated congener. Copyright © 1996 Elsevier Science Ltd

The ester-type *Cephalotaxus* alkaloids were found in *Cephalotaxus* spp., and their unique structure and potent antileukemic activities have drawn the attention of many chemists.[1] Our recent effort to search for antileukemic substances in *Cephalotaxus harringtonia* var. *drupacea* has resulted in the isolation of a novel dimeric ester alkaloid **1**, designated cephalotaxidine.

Cephalotaxidine (**1**)[2] has the molecular formula $C_{58}H_{74}N_2O_{19}$ that was established by high-resolution FABMS, indicating twenty-three degrees of unsaturation. The NMR spectral data of **1** showed the presence of four methoxy, two methylenedioxy, two AB type methylene, four ester carbonyl and four terminal methyl groups and two para-coupling aromatic rings. These data suggested that **1** was the dimer of the ester-type *Cephalotaxus* alkaloid. From the planar structure elucidated by the HMQC[3] and HMBC[4] spectra and the fragment ion from high-resolution FABMS (base peak of *m/z* 544.2518, $C_{29}H_{38}NO_9$), the A-portion of **1** appeared to have the homoharrigtonine structure.[5] For the B-portion, the HMQC and HMBC spectra gave the same planar structure as the A-portion except for the C-27 carbonyl group whose presence was further supported by the carbonyl resonance (δ_C 176.2) and the significant lower chemical shift of the C-26 resonance and the higher ones of the C-25 and the C-29 resonances. A COSY correlation was observed between the 8-H of the A-portion and the 26-H of the B-portion, and an HMBC correlation was observed between the 8-H of the A-portion and the C-25 of the B-portion (Fig. 1). Thus, these two portions were proved to be linked at the C-8 position of the A-portion and at the C-26 position of the B-portion. The NOESY correlations between 1-H and 8-H in the A-portion and between 20-H and 26-H in the B-portion established the stereochemistry of the connective site as shown in structure **1**. Other correlations



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commonly observed in both the A- and B-portions were between 3-H (22-H) and 4-H (23-H), 4-H (23-H) and 6-Hα (25-Hα), 4-H (23-H) and 14-H (33-H) and 11-Hα (30-Hα) and 17-H (36-H), which disclosed the relative stereochemistry of the two mother skeletons being the same as that of homoharringtonine. The absolute configurations of the mother skeletons of **1** were established using the circular dichromic exciton chirality method.[6] Since dibenzoate **2**, prepared from **1** through hydrolysis with LiOH and acylation with benzoyl chloride in pyridine, showed a negative Cotton effect at 228 nm with a molar ellipcity of −106100 which is almost twice the magnitude of that of cephalotaxine *O*-benzoate (**3**, $[\theta]_{228}$ −44400), the C-3 and C-22 centers were determined to be both *S* configurations (Fig. 2).[7] The absolute stereochemistry of the diacid ester side chains were determined to be both *R* configurations since the molybdate complex of the diacids derived from the acid hydrolysis of **1** showed a negative Cotton effect ($[\theta]_{266}$ −4460).[8] Thus, the absolute structure of cephalotaxidine was determined as shown in structure **1**. The IC_{50} value of **1** against P-388 leukemia cells was 1.8 μg/mL.

Alkaloids, which possess a dimeric structure, are a rare class of compounds except for the indole and the benzylisoquinoline alkaloids. This is the first example of the dimeric *Cephalotaxus* alkaloid.

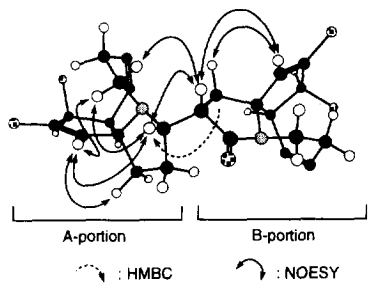

Fig. 1 Selected NOESY and HMBC correlations for **1**. The aromatic rings and the ester and ether side chains are omitted for clarity.

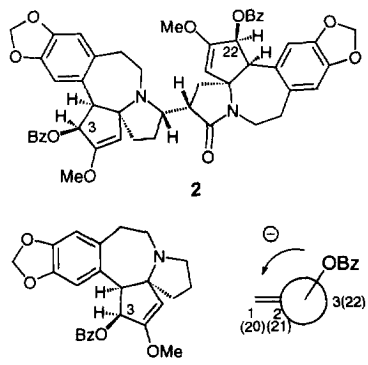

Fig. 2

Acknowledgments: We would like to thank Dr. Susumu Isoda (Showa University) for collecting and identifying the plants of *C. harringtonia* var. *drupacea*. We also thank Mr. Akihiro Shibata (Jasco International Co.) for the HR-FABMS measurements.

References and Notes

1. Huang, L.; Xue, Z. In *The alkaloids*; Brossi, A., Ed.; Academic: Orlando, **1984**; Vol. 23, pp 157–226.
2. Nine mg of **1** was isolated from 1.5 kg of the MeOH extract. Amorphous solid, $[\alpha]_D$ −172° (*c* 0.10, MeOH); HR-FABMS *m/z*: 1103.4946 $[M+H]^+$ (Calcd for $C_{58}H_{75}N_2O_{19}$: 1103.4964); ν_{max} cm^{-1}: 3380br (OH), 1736, 1649 (conj. C=O), 1490 (Ar C=C); λ_{max} (MeOH) nm: 291 (log ε 3.85); ^{1}H-NMR (500 MHz, *J*/Hz, $CDCl_3$) δ 0.82 (1H, m, 7-Hα), 1.18 (12H, s, 5"-, 5""-, 6"- and 6""-Me), 1.34–1.44 (12H, m, 1"-, 1""-, 2"-, 2""-, 3"- and 3""-Hab), 1.47 (1H, m, 25-Hβ), 1.57 (1H, m, 7-Hβ), 1.61 (1H, m, 25-Hα), 1.71 (2H, m, 6-Hα and Hβ), 1.92 (1H, d, *J* 16.3, 3'-Ha), 2.07 (1H, d, *J* 16.4, 3'"-Ha), 2.25 (1H, d, *J* 16.3, 3'-Hb), 2.32 (1H, m, 11-Hα), 2.33 (1H, d, *J* 16.4, 3'"-Hb), 2.56 (1H, m, 30-Hα), 2.61 (1H, m, 10-Hβ), 2.70 (1H, m, 10-Hα), 2.70 (1H, m, 26-H), 3.05 (1H, m, 11-Hβ), 3.08 (1H, m, 29-Hβ), 3.19 (1H, m, 30-Hβ), 3.32 (1H, m, 8-H), 3.53 (1H, d, *J* 9.4, 23-H), 3.56 (3H, s, 5'-Me), 3.57 (3H, s, 5'"-Me), 3.64 (3H, s, 38-Me), 3.66 (3H, s, 19-Me), 3.69 (1H, d, *J* 9.1, 4-H), 3.85 (1H, m, 29-Hα), 4.69 (1H, s, 20-H), 4.96 (1H, s, 1-H), 5.88 (1H, d, *J* 1.5, 18-Ha), 5.89 (1H, d, *J* 1.5, 37-Ha), 5.90 (1H, d, *J* 1.5, 37-Hb), 5.99 (1H, d, *J* 1.5, 18-Hb), 5.92 (1H, d, *J* 9.4, 22-H), 5.96 (1H, d, *J* 9.1, 3-H), 6.56 (2H, s, 14- and 33-H), 6.58 (2H, s, 17- and 36-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.8 × 2 (t, C-2" and -2""), 22.9 (t, C-7), 29.0 × 2 (q, C-5" and -5""), 29.3 × 2 (q, C-6" and -6""), 30.1 (t, C-30), 31.3 (t, C-11), 36.5 (t, C-25), 39.1 × 3 (t, C-1" -1"" and -29), 41.9 (d, C-26), 42.4 (t, C-6), 42.5 × 2 (t, C-3' and -3'"), 43.7 × 2 (t, C-3" and -3""), 45.6 (t, C-10), 51.5 × 2 (q, C-5' and -5'"), 56.6 (d, C-4), 57.4 (q, C-19), 57.6 (q, C-38), 59.1 (d, C-23), 62.8 (d, C-8), 69.7 (s, C-24), 70.8 × 2 (s, C-4" and -4""), 71.4 (s, C-5), 74.4 (d, C-3), 74.5 (d, C-22), 74.7 × 2 (s, C-2' and -2'"), 100.7 (t, C-18), 101.4 (d, C-1), 101.4 (t, C-37), 103.0 (d, C-20), 109.3 (d, C-17), 110.0 (d, C-36), 112.4 (d, C-14), 112.5 (d, C-33), 126.2 (s, C-32), 129.0 (s, C-13), 132.6 (s, C-31), 133.5 (s, C-12), 145.3 (s, C-15), 146.2 × 2 (s, C-16 and -34), 147.2 (s, C-35), 157.5 (s, C-2), 158.5 (s, C-21), 170.3 × 2 (s, C-4' and -4'"), 173.9 × 2 (s, C-1' and -1'"), 176.2 (s, C-27).
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7. **2**: $[\theta]_{293}$ −12800, $[\theta]_{276}$ 0, $[\theta]_{250}$ +25700, $[\theta]_{243}$ 0, $[\theta]_{228}$ −106100, $[\theta]_{218}$ −88500, $[\theta]_{203}$ −367000; **3**: $[\theta]_{293}$ −5400, $[\theta]_{276}$ 0, $[\theta]_{249}$ +10300, $[\theta]_{243}$ 0, $[\theta]_{228}$ −44400, $[\theta]_{217}$ −34600, $[\theta]_{203}$ −156100.
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