Isolation, Structural Elucidation and Conformational Analysis of Sesquiterpene Pyridine Alkaloids from *Maytenus ebenifolia* Reiss. X-Ray Molecular Structure of Ebenifoline W-I

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The structures of seven novel alkaloids, ebenifolines W-I, W-II, E-I, E-II, E-III, E-IV and E-V, isolated from *Maytenus ebenifolia* Reiss., were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, mainly 2D experiments, to be sesquiterpene pyridine alkaloids having either a fifteen- or sixteen-membered ring structure. The conformation of one of these alkaloids, ebenifoline W-I 1, was determined by homodecoupling experiments, NOESY spectra in NMR, and X-ray analysis. The flexibility of the two types of macrocyclic ring systems was studied by their T_1-values of ^{13}C NMR spectroscopy.

During our studies on biologically active compounds in South American medicinal plants,[1,2] we were interested in plants of the genus *Maytenus* of the Celastraceae family, widely used as folk medicines.[3,4] From *Maytenus* species, many characteristic bioactive compounds, such as maytansinoids[5] with antitumour activity, cytotoxic quinoid-type triterpenes,[1,2,6] and sesquiterpenes and sesquiterpene pyridine alkaloids[7] with insect antifeedant or insecticidal activity have been isolated. Recently, immunosuppressive activities of sesquiterpene pyridine alkaloids were also reported.[8]

In this work, from *Maytenus ebenifolia*, called 'chuchuhuasi',[3,4]† which is used for the treatment of rheumatism in Peru, we isolated twelve sesquiterpene pyridine alkaloids (four wilfordate types and eight evoninate types) including seven novel ones, and determined their structures. The core structure of these alkaloids is a dihydro-β-agarofuran sesquiterpene moiety forming a sixteen- or fifteen-membered macrocyclic ring containing two intramolecular ester linkages. In some of them, the substituent at C-2' of the 2',3'-disubstituted pyridine is 2-methylbutanoic acid[9] (wilfordate type), and in others it is 2,3-dimethylpropanoic acid[10] (evoninate type).

In the evoninate-type compounds, the configurations of the two methyl groups in the 2,3-dimethylpropanoic acid side-chain have already been determined to be 2S,3S by either hydrolysis[11] or X-ray analysis.[12] However, the configuration of the methyl group in the 2-methylbutanoic acid side-chain in the wilfordate type has not yet been determined.

The present paper is about the structural and conformational studies of the novel compounds, ebenifoline E-III, IV, V and W-II, about the configuration of the methyl group in the wildfordate-type macrocyclic moiety of ebenifoline W-I by NMR and X-ray analysis, and also on comparisons of the molecular conformational flexibilities of ebenifoline W-I and mayteine, whose macrocyclic rings differ in size.

Results and Discussion

Isolation.—A methylene dichloride-soluble portion of a methanolic extract of *Maytenus ebenifolia* Reiss. (900 g) was subjected to silica gel column chromatography. The fractions obtained were further separated by silica gel or reversed-phase silica gel medium-pressure liquid chromatography (MPLC) and/or high-performance liquid chromatography (HPLC) to give seven new sesquiterpene pyridine alkaloids, ebenifolines W-I (1: 0.0071%),‡ W-II (2: 0.0004%, E-I (5: 0.0040%), E-II (6: 0.0222%), E-III (7: 0.0037%), E-IV (8: 0.0013%) and E-V (9: 0.0014%), and also five known alkaloids, euojaponine F (3: 0.0063%),[14] euonine (4: 0.0007%),[9] euojaponine C (10: 0.0007%),[15] mayteine (11: 0.28%)[16] and euonymine (12: 0.0059%).[17]

Structure of Ebenifolines E-III 7, *IV* 8, *V* 9 *and W-II* 2.—Ebenifoline E-III was obtained as an amorphous solid with the molecular formula $C_{48}H_{51}NO_{18}$. It contained four acetyl groups (δ_H 2.04, 2.10, 2.23, 2.40), two benzoyl groups [δ_H 6.86 (*m*), 7.16 (*p*), 7.46 (*o*); 7.18 (*m*), 7.40 (*p*), 7.54 (*o*)], two tertiary methyl groups (δ_H 1.64, 1.78), two sets of methylene protons (δ_H 3.74, 5.97; 5.03, 5.29), seven methine protons (δ_H 2.43, 4.82, 5.38, 5.64, 5.68, 5.93, 7.12) and one hydrogen-bonded hydroxy group (δ_H 4.52; v 3486 cm^{-1}). It also contained one 2,3-disubstituted pyridine (δ_H 7.27, 8.09, 8.70), two secondary methyl groups (δ_H 1.23, 1.41) and two methine protons (δ_H 2.64, 4.67) which are coupled to each other. These data indicated that this was a sesquiterpene pyridine alkaloid derived from polyester sesquiterpenes, which are characteristically detected in Celastraceae plants. It included one macrocycle structure formed by two ester linkages between one sesquiterpene molecule and one evoninic acid, at positions 3 and 15. The locations of the two benzoyl groups were determined to be at positions 1 and 8 by correlation *via* long-range coupling (COLOC) spectra. The cross-peaks were observed between the carbonyl carbon at δ_C 164.76, the methine proton of position 1 at δ_H 5.93 and the *ortho* protons of the benzoyl group at δ_H 7.46, and also between the carbonyl carbon at δ_C 164.35, the methine proton of position 8 at δ_H 5.68 and the *ortho* protons of the other benzoyl group at δ_H 7.54. These two benzoyl groups were both oriented equatorially, as revealed by the coupling constants. Therefore, the structure of ebenifoline E-III was determined as 7.

Ebenifoline E-IV was an amorphous solid, with the molecular

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† Indian tribes in Peru soak the reddish dark-brown stem bark in the local 'fire water' ('auguardiente') to prepare the medicine and take it before breakfast for the treatment of rheumatism.

‡ Structural elucidation of these alkaloids has already been reported in a preliminary form (see ref. 13).

formula $C_{43}H_{49}NO_{17}$. The NMR spectrum suggested that it was an evoninate-type sesquiterpene pyridine alkaloid having five acetyl groups (δ_H 1.42, 2.13, 2.14, 2.21, 2.27) and one benzoyl group [δ_H 7.40 (m), 7.53 (p), 7.87 (o)]. One secondary methyl group (δ_H 1.24) on the sesquiterpene part, coupled with one methine proton (δ_H 2.78), but there was no hydrogen-bonded hydroxy group. The benzoyl group, bonded on the ses-quiterpene structure, gave an anisotropic effect to one acetyl group (δ_H 1.42), as shown by 2D heteronuclear multiple bond coherence (HMBC) [18] spectra. The methine proton (δ_H 6.04) of position 1 and the *ortho* protons (δ_H 7.87) of the benzoyl group gave cross-peaks with the carbonyl carbon at δ_C 164.69, and the methine proton (δ_H 5.39) at position 8 and the acetyl methyl group (δ_H 1.42) also gave cross-peaks with the carbonyl carbon at δ_C 168.96. These data showed that the benzoyl group and the acetyl group were at positions 1 and 8, respectively, and that the structure of ebenifoline E-IV was 4-deoxymayteine.
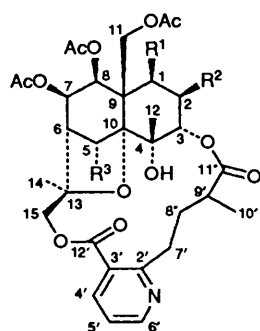
R¹   R²   R³

ebenifoline W-I   1   OCOPh   OCOPh   OAc
ebenifoline W-II 2   OCOPh   OCOPh   OH
euojaponine F   3   OCOPh   OAc   OAc
euonine   4   OAc   OAc   OAc
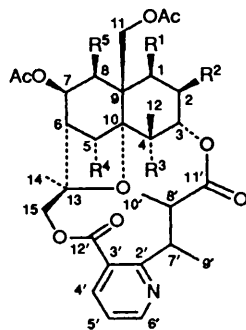
R¹   R²   R³   R⁴   R⁵

ebenifoline E-I   5   OCOPh   OH   OH   OAc   OAc
ebenifoline E-II   6   OCOPh   OAc   OH   OCOPh OAc
ebenifoline E-III 7   OCOPh   OAc   OH   OAc   OCOPh
ebenifoline E-IV   8   OCOPh   OAc   H   OAc   OAc
ebenifoline E-V   9   OCOPh   OAc   OH   OH   OCOPh
euojaponine C   10   OCOPh   OH   OH   OCOPh OAc
mayteine   11   OCOPh   OAc   OH   OAc   OAc
euonymine   12   OAc   OAc   OH   OAc   OAc

Ebenifoline E-V, an amorphous solid with the molecular formula $C_{46}H_{49}NO_{17}$, was an evoninate-type sesquiterpene pyridine alkaloid containing three acetyl groups (δ_H 2.04, 2.10, 2.26), two benzoyl groups [δ_H 6.86 (m), 7.17 (p), 7.48 (o); 7.19 (m), 7.41 (p), 7.51 (o)]. HMBC spectra showed that the non-carbon bonded protons at δ_H 5.74 and 6.07 gave cross-peaks with the carbons at δ_C 23.53 (C-12) and 72.65 (C-4), and the carbons at δ_C 74.50 (C-5) and 93.16 (C-10), respectively. Therefore there are two free hydroxy groups located at positions 4 and 5. Analogously, two benzoyl groups were shown to be located at positions 1 and 8, by the cross-peaks between 1-H (δ_H
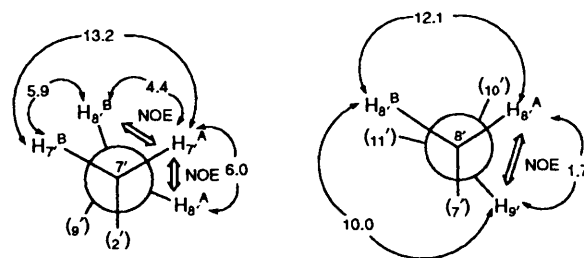

Fig. 1   Coupling constants (J/Hz) and NOE relationships around C-7' and 8' of ebenifoline W-I 1

5.95), Bz o-H (δ_H 7.48) and the carbonyl carbon at δ_C 164.99, and between 8-H (δ_H 5.68), Bz o-H (δ_H 7.51) and the carbonyl carbon at δ_C 164.42. The structure of ebenifoline E-V was determined as 9.

Ebenifoline W-II was an amorphous solid which had the molecular formula $C_{46}H_{49}NO_{17}$. It was a sesquiterpene pyridine alkaloid containing three acetyl groups (δ_H 1.35, 1.94, 2.12), two benzoyl groups [δ_H 7.31 (m), 7.49 (p), 7.79 (o); 7.51 (m), 7.63 (p), 8.03 (o)], two sets of methylenes coupled to each other (δ_H 1.9, 2.4; 2.91, 4.12), one secondary methyl group (δ_H 1.25) and one methine proton (δ_H 2.4). These data indicated that its nicotinate derivative unit in the macrocycle was wilfordic acid. There were two hydroxy groups in the sesquiterpene part as in ebenifoline E-V, and their positions were determined to be positions 4 and 5, by the cross-peaks between the hydroxy group at δ_H 6.40, C-12 at δ_C 23.92 and C-4 at δ_C 71.96, and between the hydroxy group at δ_H 6.03 and C-10 at δ_C 92.97. The positions of two benzoyl groups were determined to be at positions 1 and 2 by the presence of the cross-peaks between 1-H at δ_H 6.15, Bz o-H at δ_H 7.79 and the carbonyl carbon at δ_C 165.33, and between 2-H at δ_H 5.55, Bz o-H at δ_H 8.03 and the carbonyl carbon at δ_C 164.89. These data showed that the structure of ebenifoline W-II was 2.

The complete assignments of the ¹H and ¹³C NMR signals of all ebenifolines are shown in Tables 1 and 2, respectively. Other isolated compounds, euojaponines C and F, euonine, mayteine and euonymine were identified by spectral analysis, listed in the Experimental section. Of the compounds isolated from this material in this work, mayteine was the major component, the yield of which was 0.28% by weight of dry plant (~2.5 g from 900 g).

Configuration and Conformation of Wilfordate-type Alka-loids.—Forty to fifty macrocyclic sesquiterpene pyridine alka-loids have been isolated from the Celastraceae. These com-pounds may be roughly divided into two types, one (evoninate type) containing evoninic acid in the macrocycle moiety, and the other (wilfordate type) containing wilfordic acid in the macrocycle moiety. (Other types whose macrocycle structures include cassinic acid,[19] hydroxywilfordic acid,[20] isoevoninic acid,[21] isowilfordic acid,[22] etc. are also known.) The con-figurations of the methyl groups of the nicotinate derivative moiety of the evoninate-type compounds were determined as 7'S,8'S (evoninic acid: 2S,3S) by the results of hydrolysis[11] and X-ray analysis.[12] However, the configuration of the methyl group of the wilfordic acid moiety at position 9' has not been determined. In this work, the configuration of the methyl group was studied by using coupling constants and space-distance relationships by means of NMR homodecoupling experiments and 2D nuclear Overhauser enhancement (NOESY) spectra, and comparison with those of ebenifoline W-I 1 in whose ¹H NMR spectrum the signals caused by the side-chain com-ponents of the nicotinate derivative unit were less overlapping. In the NOESY spectra, cross-peaks were observed between 7'-Hᴬ and 8'-Hᴬ and 8'-Hᴮ, and also between 9'-H and 8'-Hᴬ.

Table 1 (a) ^{1}H NMR chemical shifts^a for ebenifoline W series (wilfordate-type) alkaloids

Proton	Ebenifoline W-I 1	Ebenifoline W-II 2
1-H	6.13 (d, 3, 3.9)	6.15 (d, 3.6)
2-H	5.52 (dd, 2.4, 3.9)	5.55 (dd, 2.6, 3.6)
3-H	5.16 (d, 2.4)	5.21 (d, 2.6)
4-OH	4.99 (s)	6.40 (s)
5-H	6.96 (s)	5.37 (s)
6-H	2.40 (d, 3.8)	2.47 (d, 3.9)
7-H	5.57 (dd, 3.8, 5.8)	5.54 (dd, 3.9, 5.6)
8-H	5.48 (d, 5.8)	5.44 (d, 5.6)
11-H^A	4.61 (d, 13.3)	4.67 (d, 13.0)
11-H^B	5.69 (d, 13.3)	5.56 (d, 13.0)
12-H$_3$	1.70 (s)	2.01 (d, 0.8)
14-H$_3$	1.74 (s)	1.73 (s)
15-H^A	3.83 (d, 11.9)	3.76 (d, 12.4)
15-H^B	5.77 (d, 11.9)	5.90 (d, 12.4)
4'-H	8.34 (dd, 1.6, 8.0)	8.39 (dd, 1.8, 8.0)
5'-H	7.30 (dd, 4.7, 8.0)	7.30 (dd, 4.7, 8.0)
6'-H	8.77 (dd, 1.6, 4.7)	8.78 (dd, 1.8, 4.7)
7'-H^A	2.99 (ddd, 4.4, 6.0, 13.2)	2.91 (m)
7'-H^B	3.97 (ddd, 5.9, 9.2, 13.2)	4.12 (ddd, 4.8, 11.0, 12.9)
8'-H^A	2.04 (dddd, 1.7, 6.0, 9.2, 12.1)	1.9 (m)
8'-H^B	2.32 (dddd, 4.4, 5.9, 10.0, 12.1)	2.4 (m)
9'-H	2.47 (dqd, 1.7, 6.9, 10.0)	2.4 (m)
10'-H$_3$	1.26 (d, 6.9)	1.25 (d, 6.5)
1-OAc		
OBz(o)	7.78 (d, 7.7)	7.79 (dd, 1.3, 7.8)
OBz(m)	7.32 (t-like, 7.8)	7.31 (t-like, 7.5)
OBz(p)	7.49 (t-like, 7.9)	7.49 (t-like, 7.4)
2-OAc		
OBz(o)	8.09 (d, 7.7)	8.03 (dd, 1.3, 7.9)
OBz(m)	7.51 (t-like, 7.8)	7.51 (t-like, 7.6)
OBz(p)	7.63 (t-like, 7.4)	7.63 (t-like, 7.4)
5-OAc	2.19 (s)	6.03 (br s; 5-OH)
7-OAc	2.14 (s)	2.12 (s)
8-OAc	1.36 (s)	1.35 (s)
11-OAc	2.21 (s)	1.94 (s)

(b) ^{1}H NMR chemical shifts^a for ebenifoline E series (evoninate-type) alkaloids

Proton	Ebenifoline E-I 5	Ebenifoline E-II 6	Ebenifoline E-III 7	Ebenifoline E-IV 8	Ebenifoline E-V 9
1-H	5.76 (d, 3.9)	5.94 (d, 3.9)	5.93 (d, 4.1)	6.04 (d, 4.0)	5.95 (d, 3.9)
2-H	4.13 (dd, 2.6, 3.9)	5.41 (dd, 2.5, 3.9)	5.38 (dd, 2.4, 4.1)	5.46 (ddd, 1.2, 2.2, 4.0)	5.42 (dd, 2.6, 3.9)
3-H	4.83 (d, 2.6)	4.83 (d, 2.5)	4.82 (d, 2.4)	4.86 (dd, 1.3, 2.2)	4.82 (d, 2.6)
4-OH	4.47 (d, 1.2)	4.78 (br s)	4.52 (d, 0.9)	2.78 (br q, 7.9; 4-H)	5.74 (d, 1.3)
5-H	7.06 (s)	7.22 (s)	7.12 (s)	6.76 (s)	5.56 (br s)
6-H	2.35 (d, 4.0)	2.55 (d, 4.0)	2.43 (d, 3.9)	2.44 (d, 3.8)	2.53 (dd, 0.6, 3.8)
7-H	5.53 (dd, 4.0, 5.8)	5.58 (dd, 4.0, 5.7)	5.64 (dd, 3.9, 6.0)	5.54 (dd, 3.8, 5.8)	5.63 (dd, 3.8, 5.9)
8-H	5.41 (d, 5.8)	5.48 (d, 5.7)	5.68 (d, 6.0)	5.39 (d, 5.8)	5.68 (d, 5.9)
11-H^A	4.81 (d, 13.8)	4.76 (d, 13.4)	5.03 (d, 13.6)	4.69 (d, 13.2)	5.05 (d, 13.4)
11-H^B	5.47 (d, 13.8)	5.41 (d, 13.4)	5.29 (d, 13.6)	5.31 (d, 13.2)	5.24 (d, 13.4)
12-H$_3$	1.63 (d, 1.2)	1.61 (s)	1.64 (d, 0.9)	1.24 (d, 7.9)	1.92 (d, 1.3)
14-H$_3$	1.71 (s)	1.75 (s)	1.78 (s)	1.55 (s)	1.78 (s)
15-H^A	3.69 (d, 11.7)	3.64 (d, 11.5)	3.74 (d, 11.5)	4.02 (d, 11.1)	3.74 (d, 12.1)
15-H^B	5.98 (d, 11.7)	6.05 (d, 11.5)	5.97 (d, 11.5)	5.14 (br d, 11.1)	6.10 (dd, 0.7, 12.1)
4'-H	8.06 (dd, 1.8, 7.8)	8.07 (dd, 1.7, 7.8)	8.09 (dd, 1.8, 7.8)	7.99 (dd, 1.8, 7.8)	8.16 (dd, 1.8, 7.8)
5'-H	7.26 (dd, 4.9, 7.8)	7.27 (dd, 4.8, 7.8)	7.27 (dd, 4.8, 7.8)	7.24 (dd, 4.8, 7.8)	7.29 (dd, 4.9, 7.8)
6'-H	8.69 (dd, 1.8, 4.9)	8.71 (dd, 1.7, 4.8)	8.70 (dd, 1.8, 4.8)	8.68 (dd, 1.8, 4.8)	8.71 (dd, 1.8, 4.9)
7'-H	4.67 (q, 7.0)	4.74 (q, 6.9)	4.67 (q, 7.0)	4.12 (dq, 1.1, 7.0)	4.83 (br q, 7.0)
8'-H	2.58 (q, 7.1)	2.64 (q, 7.1)	2.64 (q, 7.1)	2.83 (dq, 1.1, 7.3)	2.61 (dq, 0.8, 7.1)
9'-H$_3$	1.38 (d, 7.0)	1.46 (d, 6.9)	1.41 (d, 7.0)	1.43 (d, 7.0)	1.43 (d, 7.0)
10'-H$_3$	1.17 (d, 7.1)	1.24 (d, 7.1)	1.23 (d, 7.1)	1.31 (d, 7.3)	1.20 (d, 7.1)
1-OBz(o)	7.95 (dd, 1.2, 8.5)	7.84 (d, 7.3)	7.46 (dd, 0.9, 8.1)	7.87 (dd, 1.2, 8.2)	7.48 (dd, 1.4, 8.4)
OBz(m)	7.42 (t-like, 7.8)	7.40 (t-like, 7.7)	6.86 (t-like, 7.8)	7.40 (dt-like, 1.5, 7.7)	6.86 (t-like, 7.9)
OBz(p)	7.56 (dt-like, 1.3, 7.5)	7.54 (t-like, 7.4)	7.16 (dt-like, 0.9, 7.4)	7.53 (tt-like, 1.4, 7.4)	7.17 (dt-like, 1.3, 7.5)
2-OAc		2.17 (s)	2.10 (s)	2.14 (s)	2.10 (s)
5-OAc	2.21 (s)		2.23 (s)	2.21 (s)	6.07 (d, 2.9; 5-OH)
OBz(o)		8.34 (d, 7.3)			
OBz(m)		7.50 (t-like, 7.7)			
OBz(p)		7.60 (t-like, 7.4)			
7-OAc	2.11 (s)	2.21 (s)	2.04 (s)	2.13 (s)	2.04 (s)
8-OAc	1.40 (s)	1.43 (s)		1.42 (s)	
OBz(o)			7.54 (dd, 1.2, 7.7)		7.51 (dd, 1.4, 8.3)
OBz(m)			7.18 (t-like, 7.8)		7.19 (t-like, 7.8)
OBz(p)			7.40 (dt-like, 1.2, 7.4)		7.41 (tt-like, 1.2, 7.5)
11-OAc	2.37 (s)	2.37 (s)	2.40 (s)	2.27 (s)	2.26 (s)

^{a} Measurements performed in CDCl$_3$ at 400 MHz. Multiplicity and coupling constants (J/Hz) given in parenthesis.

Table 2 ^{13}C NMR chemical shifts[a] for ebenifolines

Carbon	1	2	5	6	7	8	9
1	73.47 (d)	73.54 (d)	75.49 (d)	73.51 (d)	73.61 (d)	74.36 (d)	73.52 (d)
2	70.51 (d)	70.66 (d)	70.11 (d)	69.21 (d)	69.26 (d)	70.03 (d)	69.09 (d)
3	75.94 (d)	75.20 (d)	78.39 (d)	75.86 (d)	75.77 (d)	74.02 (d)	75.18 (d)
4	69.88 (s)	71.96 (s)	70.64 (s)	70.72 (s)	70.70 (s)	35.99 (d)	72.65 (s)
5	73.85 (d)	74.28 (d)	74.07 (d)	74.89 (d)	73.98 (d)	74.41 (d)	74.50 (d)
6	51.18 (d)	52.56 (d)	50.52 (d)	50.40 (d)	50.53 (d)	50.41 (d)	51.89 (d)
7	69.03 (d)	69.34 (d)	69.07 (d)	69.11 (d)	69.26 (d)	69.20 (d)	69.44 (d)
8	71.65 (d)	71.96 (d)	71.78 (d)	71.55 (d)	71.55 (d)	71.76 (d)	71.76 (d)
9	52.54 (s)	51.21 (s)	53.00 (s)	52.72 (s)	53.23 (s)	51.27 (s)	52.05 (s)
10	93.89 (s)	92.97 (s)	94.62 (s)	93.83 (s)	94.24 (s)	90.84 (s)	93.16 (s)
11	60.83 (t)	61.63 (t)	60.47 (t)	60.15 (t)	60.17 (t)	60.12 (t)	60.97 (t)
12	23.15 (q)	23.92 (q)	23.13 (q)	22.91 (q)	23.12 (q)	14.19 (q)	23.53 (q)
13	84.71 (s)	85.44 (s)	84.24 (s)	84.44 (s)	84.45 (s)	82.60 (s)	85.03 (s)
14	17.97 (q)	18.21 (q)	18.47 (q)	18.35 (q)	18.55 (q)	18.18 (q)	18.80 (q)
15	70.39 (t)	71.35 (t)	70.17 (t)	70.04 (t)	69.99 (t)	69.89 (t)	70.88 (t)
2'	164.09 (s)	165.21 (s)	165.21 (s)	165.23 (s)	165.38 (s)	163.70 (s)	165.93 (s)
3'	124.09 (s)	124.04 (s)	125.45 (s)	125.20 (s)	125.19 (s)	126.74 (s)	124.55 (s)
4'	138.78 (d)	138.78 (d)	137.72 (d)	137.66 (d)	137.81 (d)	137.93 (d)	138.29 (d)
5'	121.20 (d)	121.31 (d)	121.17 (d)	121.14 (d)	121.17 (d)	121.13 (d)	121.32 (d)
6'	153.21 (d)	153.63 (d)	151.49 (d)	151.51 (d)	151.57 (d)	151.15 (d)	151.80 (d)
7'	33.49 (t)	33.15 (t)	36.58 (d)	36.48 (d)	36.63 (d)	37.46 (d)	36.24 (d)
8'	33.35 (t)	33.58 (t)	45.08 (d)	45.05 (d)	45.00 (d)	43.69 (d)	45.49 (d)
9'	38.55 (d)	38.33 (d)	12.05 (q)	11.90 (d)	12.03 (q)	12.70 (q)	11.53 (q)
10'	18.82 (q)	19.12 (q)	9.48 (q)	9.72 (q)	9.87 (q)	10.53 (q)	9.75 (q)
11'	175.12 (s)	175.32 (s)	174.71 (s)	173.93 (s)	174.04 (s)	175.63 (s)	173.86 (s)
12'	166.94 (s)	167.21 (s)	168.57 (s)	168.61 (s)	168.58 (s)	167.94 (s)	168.97 (s)
1-OC=O	165.02 (s)	165.33 (s)	164.98 (s)	164.62 (s)	164.76 (s)	164.69 (s)	164.99 (s)
ipso	129.33 (s)	128.91 (s)	129.35 (s)	129.21 (s)	128.40 (s)	129.41 (s)	128.56 (s)
ortho	129.62 (d)	129.68 (d)	129.81 (d)	129.58 (d)	129.22 (d)	129.61 (d)	129.29 (d)
meta	128.42 (d)	128.37 (d)	128.67 (d)	128.52 (d)	127.82 (d)	128.60 (d)	127.83 (d)
para	133.38 (d)	133.33 (d)	133.68 (d)	133.48 (d)	132.88 (d)	133.50 (d)	132.86 (d)
2-OC=O	164.85 (s)	164.89 (s)		165.23 (s)	168.16 (s)	168.67 (s)	168.09 (s)
Me				20.88 (q)	20.91 (q)	20.97 (q)	20.92 (q)
ipso	128.85 (s)	129.68 (s)					
ortho	129.96 (d)	129.85 (d)					
meta	128.83 (d)	128.91 (d)					
para	133.79 (d)	133.84 (d)					
5-OC=O	169.93 (s)		169.99 (s)	165.79 (s)	169.96 (s)	169.64 (s)	
Me	21.61 (q)		21.71 (q)		21.68 (q)	21.50 (q)	
ipso				129.52 (s)			
ortho				130.34 (d)			
meta				128.83 (d)			
para				133.58 (d)			
7-OC=O	170.02 (s)	169.94 (s)	170.11 (s)	170.06 (s)	169.80 (s)	170.01 (s)	169.64 (s)
Me	20.99 (q)	21.00 (q)	21.02 (q)	21.03 (q)	20.97 (q)	21.03 (q)	21.02 (q)
8-OC=O	168.96 (s)	168.93 (s)	169.12 (s)	168.90 (s)	164.35 (s)	168.96 (s)	164.42 (s)
Me	19.88 (q)	19.91 (q)	20.01 (q)	19.90 (q)		19.95 (q)	
ipso					128.89 (s)		129.04 (2)
ortho					129.28 (d)		129.31 (d)
meta					128.32 (d)		128.29 (d)
para					133.10 (d)		133.08 (d)
11-OC=O	170.47 (s)	170.11 (s)	170.28 (s)	170.26 (s)	170.50 (s)	170.31 (s)	170.26 (s)
Me	21.23 (q)	21.12 (q)	21.52 (q)	21.47 (q)	21.53 (q)	21.44 (q)	21.74 (q)

[a] Measurements performed in CDCl₃ at 100 MHz. Multiplicity given in parenthesis.
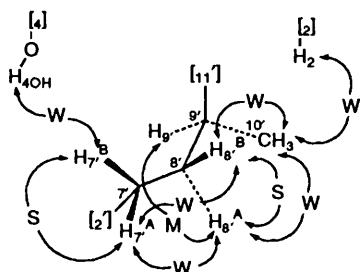
Fig. 2 Conformation of the side chain in ebenifoline W-I 1. ←→ NOE relationship (W; weak, M; medium, S; strong).

Moreover, the coupling constants and the NOE data given in Fig. 1 show the relationships between the C-7' methylene protons and the C-8' methylene protons, and also between the C-8' methylene protons and the C-9' methine proton. The

cross-peaks between the C-10' methyl group and the methine proton at position 2 of the sesquiterpene core, and between 7'-H[B] proton and the proton of the hydrogen-bonded hydroxy group at position 4 revealed the conformation of the wilfordate side-chain to be as shown in Fig. 2. In this manner, the configuration at position 9', which has not previously been known, was assigned as S.

X-Ray Analysis of Ebenifoline W-I 1.*—In order to confirm the configuration at C-9', a single plate crystal of ebenifoline W-I 1 from EtOH–MeOH–water was subjected to X-ray analysis. The result, shown in Fig. 3, confirmed the configuration estimated by the NMR spectra. The X-ray analysis

* The absolute configuration of this compound has already been determined by application of the CD exciton chirality method (see ref. 13).
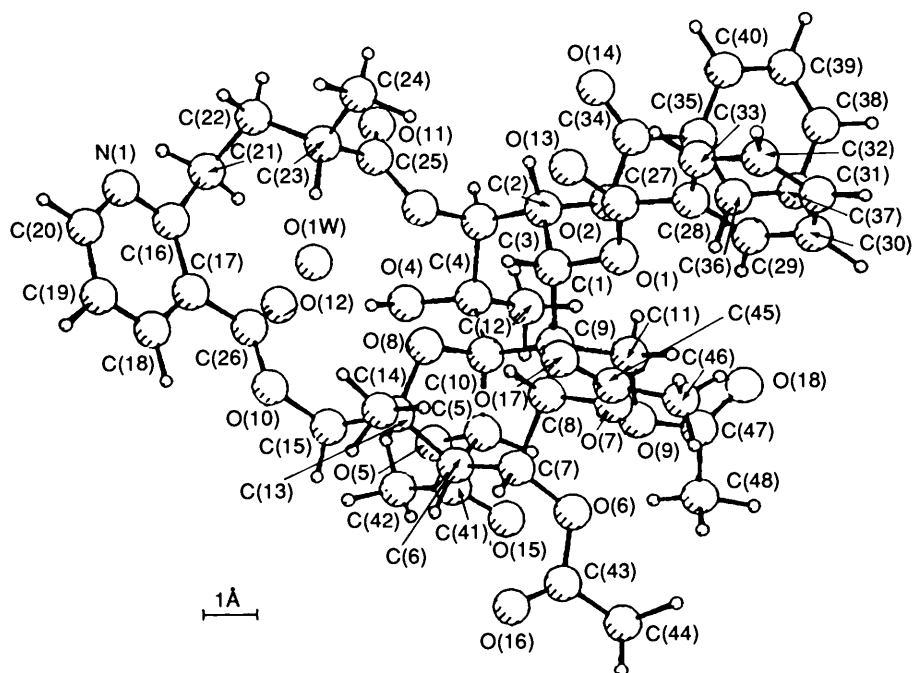
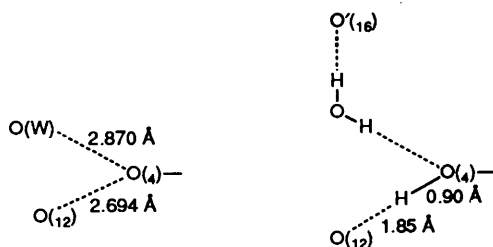**Fig. 3** Molecular structure of ebenifoline W-I **1** by X-ray analysis

Fig. 4 Hydrogen bonds around 4-OH in compound **1**

revealed also the presence of two hydrogen bonds, the only proton-donor being O(4) (in hydroxy group): O(4)···O(12) (2.694 Å) and O(4)···O(1W) (water of crystallization; 2.870 Å). By refinements of the hydrogen atom by a difference Fourier synthesis, the hydrogen atom O(4)–H was shown to be substantially oriented towards O(12) as shown in Fig. 4, to form an intramolecular hydrogen bond O(4)–H ··· O(12). The water molecule, one of whose hydrogens forms a hydrogen bond with O(4) [O(4) ··· O(1W)] also forms another hydrogen bond with the carbonyl oxygen O(16) of another molecule.

Flexibility of Macrocyclic Ring System.—The evoninate-type and the wilfordate-type sesquiterpene pyridine alkaloids possess either a fifteen- or sixteen-membered macrocyclic ring structure in their molecule. The effect of the difference in their ring sizes on the stability of the macrocyclic ring system was studied by evaluating the flexibilities of these ring systems by measuring the spin-lattice relaxation time, T_1, by ^{13}C NMR spectroscopy. In this experiment, mayteine **11**, an evoninate-type compound with a fifteen-membered ring, obtainable in large amounts, and ebenifoline W-I **1**, a wilfordate-type compound with a sixteen-membered ring, were used. The NT_1-values, in which N is the number of protons attached to a particular carbon atom and T_1 is the longitudinal relaxation time, are given in Fig. 5. In the fifteen-membered macrocyclic ring compound, mayteine, the flexibilities of the carbons around the two ester linkages joining the sesquiterpene skeleton with the nicotinate derivative, *i.e.* the C-14 methyl group, C-15 methylene group and C-3 methine group were slightly higher than those of the corresponding groups of the sixteen-
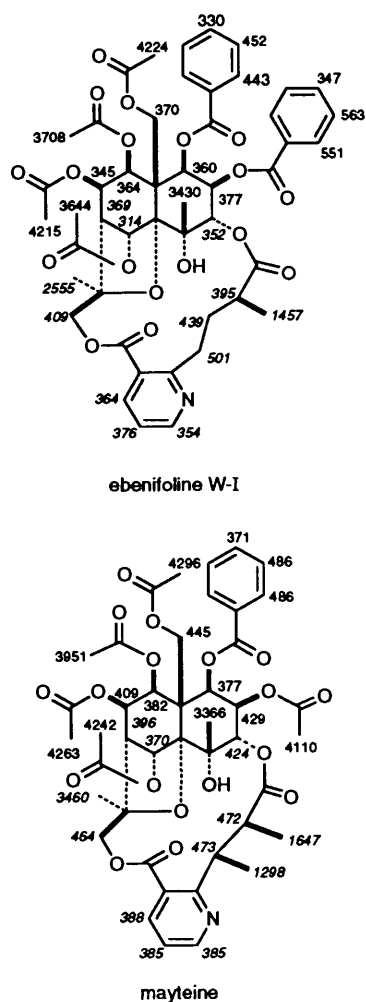

ebenifoline W-I

mayteine

Fig. 5 NT_1-Values (ms) for ebenifoline W-I **1** and mayteine **11**. Measurements performed at 23.5 mmol/0.5 cm³ in CDCl₃ at 100 MHz. NT_1-Values were N times T_1-values in which N = the number of attached protons and T_1 = longitudinal relaxation time.

membered-ring compound, ebenifoline W-I. It is interesting that two methyl groups of mayteine have different flexibilities. Since the NT_1-values of the side-chains of the nicotinate derivative moiety of these two compounds were similar, and were only slightly larger than those of the sesquiterpene skeletons, these macrocyclic ring structures were considered to be moderately rigid and their rigidity was considered to be caused by the intramolecular hydrogen bond between the C-4 hydroxy group and the C-12' carbonyl carbon, which restricted the flexibilities of their macrocyclic ring conformations significantly. No significant difference in flexibility was observed between the fifteen- and the sixteen-membered rings, though the results suggest slightly higher flexibilities in fifteen-membered rings compared with sixteen-membered ones.

Experimental

General Details.—M.p.s were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 spectrometer and the $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. Mass spectra, UV and IR spectra were taken with a Hitachi M-80 spectrometer, a Hitachi 557 spectrophotometer and a Perkin-Elmer 1710 spectrophotometer, respectively. MPLC was performed with a CIG column system (22 mm i.d. × 300 mm, Kusano Scientific Co., Tokyo) packed with 10 µm silica gel or 20 µm ODS. HPLC was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, Gasukuro Kogyo Inc.) packed with 10 µm ODS. TLC was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by heating after spraying with 10% H_2SO_4. ^{1}H and ^{13}C NMR spectra were recorded on Bruker spectrometers (AM400 and AM500) at 303 K and processed on a Bruker data station with an Aspect 3000 computer. Phase sensitive 2D nuclear Overhauser enhancement (NOESYPH) experiments were made with a mixing time of 0.6 s. The NMR coupling constants (J) are given in Hz.

Materials.—Reddish dark-brown stem bark of *Maytenus ebenifolia* Reiss. (900 g), commonly known as 'chuchuhuasi' among Indian tribes, was purchased at Iquitos, Peru in 1990. The botanical identification was made by Dr. F. Ayala Flores, director of Amazonese Herbarium, Universidad Nacional de la Amazonia Peruana, Iquitos, Peru.

Extraction and Isolation of Alkaloids 1–12.—Dry bark (900 g) of *M. ebenifolia* was extracted with methanol to give a methanol extract (268 g) which was partitioned between methylene dichloride and water. The methylene dichloride-soluble fraction (24 g) was subjected to silica gel column chromatography with a methylene dichloride–ethyl acetate gradient system (1:0–0:1) to give 14 fractions. Fractions 6–8 were further subjected to silica gel MPLC with a hexane–ethyl acetate solvent system, followed by ODS MPLC with a methanol–water solvent system to give compound 11 in a large amount, and compounds 7–10 as amorphous solids. By analogous silica gel MPLC, followed by ODS MPLC, fraction 9 gave compounds 1, 5, 6, 10, 11 and 12 as amorphous solids, and fractions 10 and 11 gave 1–4 as amorphous solids. These compounds were further purified by ODS HPLC with methanol–water or acetonitrile–water solvent systems.

Ebenifoline W-I 1.—Plates, m.p. 222–224 °C; $[\alpha]_D$ + 47.1 (*c* 0.29, CHCl₃); λ_{max}(EtOH)/nm ($\Delta\varepsilon$) 240 (+22.6) and 223 (−15.1); *m/z* 929 (M⁺), 870, 812, 572, 206, 178, 161 and 105 (Found: M⁺, 929.3124. C₄₈H₅₁NO₁₈ requires *M*, 929.3143); ν_{max}(CHCl₃)/cm¹ 3444 and 1735 m; λ_{max}(EtOH)/nm (log ε) 202

(4.55), 229 (4.48) and 266 (3.72); δ_H(CDCl₃) see Table 1; δ_C(CDCl₃) see Table 2.

Ebenifoline W-II 2.—Amorphous solid, m.p. 118–122 °C; $[\alpha]_D$ + 61.1 (*c* 0.18, CHCl₃); λ_{max}(EtOH)/nm ($\Delta\varepsilon$) 239 (+28.7) and 222 (−21.3); *m/z* 887 (M⁺), 828, 814, 756, 530, 262, 206, 178 and 105 (Found: M⁺, 887.3037. C₄₆H₄₉NO₁₇ requires *M*, 887.2999); ν_{max}(CHCl₃)/cm⁻¹ 3450 and 1730; λ_{max}(EtOH)/nm (log ε) 203 (4.62), 228 (4.71) and 267 (3.93); δ_H(CDCl₃) see Table 1; δ_C(CDCl₃) see Table 2.

Euojaponine F 3.—Amorphous solid, m.p. 148–150 °C; $[\alpha]_D$ + 8.3 (*c* 1.06, CHCl₃); *m/z* 867 (M⁺), 866, 808, 750, 572, 262, 206, 178, 161 and 105; ν_{max}(CHCl₃)/cm⁻¹ 3446 and 1746 m; λ_{max}(EtOH)/nm (log ε) 201 (4.25), 228 (4.14) and 268 (3.47); δ_H(CDCl₃) 1.21 (3 H, d, 7.0, 10'-H₃), 1.42 (3 H, s, 8-OAc), 1.59 (3 H, d, 1.1, 12-H₃), 1.70 (3 H, s, 14-H₃), 2.0 (1 H, m, 8'-Hᴬ), 2.14 (3 H, s, 7-OAc), 2.15 (3 H, s, 2-OAc), 2.19 (3 H, s, 5-OAc), 2.32 (1 H, m, 8'-Hᴮ), 2.32 (3 H, s, 11-OAc), 2.37 (1 H, d, 3.9, 6-H), 2.41 (1 H, m, 9'-H), 2.97 (1 H, m, 7'-Hᴬ), 3.80 (1 H, d, 11.9, 15-Hᴬ), 3.94 (1 H, ddd, 6.3, 9.7 and 13.5, 7'-Hᴮ), 4.66 (1 H, d, 13.4, 11-Hᴬ), 4.95 (1 H, d, 1.1, 4-OH), 5.01 (1 H, d, 2.7, 3-H), 5.25 (1 H, dd, 2.7 and 3.7, 2-H), 5.41 (1 H, d, 13.4, 11-Hᴮ), 5.43 (1 H, d, 5.7, 8-H), 5.54 (1 H, dd, 3.9 and 5.7, 7-H), 5.75 (1 H, d, 11.9, 15-Hᴮ), 5.97 (1 H, d, 3.7, 1-H), 6.96 (1 H, s, 5-H), 7.28 (1 H, dd, 4.7 and 8.0, 5'-H), 7.40 (2 H, dt-like, 1.5 and 7.8, OBz *m*-H), 7.54 (1 H, dt-like, 1.3 and 7.4, OBz *p*-H), 7.84 (2 H, dd, 1.3 and 8.4, OBz *o*-H), 8.33 (1 H, dd, 1.8 and 8.0, 4'-H), 8.76 (1 H, dd, 1.8 and 4.7, 6'-H); δ_C(CDCl₃) 17.94 (q, C-14), 18.83 (q, C-10'), 19.98 (q, 8-OCO*Me*), 20.94 (q, 2-OCO*Me*), 21.01 (q, 7-OCO*Me*), 21.44 (q, 11-OCO*Me*), 21.64 (q, 5-OCO*Me*), 22.77 (q, C-12), 33.36 (t, C-8'), 33.52 (t, C-7'), 38.54 (d, C-9'), 51.17 (d, C-6), 52.62 (s, C-9), 60.36 (t, C-11), 69.08 (d, C-7), 69.94 (d, C-2), 69.98 (s, C-4), 70.42 (t, C-15), 71.72 (d, C-8), 73.61 (d, C-1), 73.92 (d, C-5), 75.97 (d, C-3), 84.69 (s, C-13), 93.91 (s, C-10), 121.21 (d, C-5'), 124.49 (s, C-3'), 128.55 (2d, Bz C-*m*), 129.37 (s, Bz C-*i*), 129.58 (2d, Bz C-*o*), 133.50 (d, Bz C-*p*), 138.78 (d, C-4'), 153.25 (d, C-6'), 164.12 (s, C-2'), 164.89 (s, 1-OCO), 166.95 (s, C-12'), 168.46 (s, 2-OCO), 168.99 (s, 8-OCO), 169.93 (s, 5-OCO), 170.05 (s, 7-OCO), 170.41 (s, 11-OCO) and 175.20 (s, C-11').

Euonine 4.—Amorphous solid, m.p. 144–148 °C; $[\alpha]_D$ − 2.9 (*c* 0.43, CHCl₃); *m/z* 805 (M⁺), 747, 688, 572, 269, 219, 206, 178 and 119; ν_{max}(CHCl₃)/cm⁻¹ 3451 and 1751 m; λ_{max}(EtOH)/nm (log ε) 201 (4.08), 225 (3.87) and 269 (3.50); δ_H(CDCl₃) 1.18 (3 H, d, 6.9, 10'-H₃), 1.55 (3 H, d, 1.1, 12-H₃), 1.65 (3 H, s, 14-H₃), 1.86 (3 H, s, 1-OAc), 1.9 (1 H, m, 8'-Hᴬ), 1.99 (3 H, s, 8-OAc), 2.14 (3 H, s, 2-OAc), 2.17 (3 H, s, 5-OAc), 2.17 (3 H, s, 7-OAc), 2.28 (1 H, m, 8'-Hᴮ), 2.28 (3 H, s, 11-OAc), 2.34 (1 H, d, 3.8, 6-H), 2.36 (1 H, m, 9'-H), 2.95 (1 H, ddd, 5.8, 5.9 and 13.5, 7'-Hᴬ), 3.77 (1 H, d, 11.9, 15-Hᴬ), 3.92 (1 H, ddd, 6.1, 9.8 and 13.5, 7'-Hᴮ), 4.46 (1 H, d, 13.4, 11-Hᴬ), 4.90 (1 H, br s, 4-OH), 4.94 (1 H, d, 2.7, 3-H), 5.16 (1 H, dd, 2.7 and 3.6, 2-H), 5.23 (1 H, d, 13.4, 11-Hᴮ), 5.36 (1 H, d, 5.9, 8-H), 5.52 (1 H, dd, 3.8 and 5.9, 7-H), 5.62 (1 H, d, 3.6, 1-H), 5.73 (1 H, d, 11.9, 15-Hᴮ), 6.91 (1 H, s, 5-H), 7.27 (1 H, dd, 4.8 and 8.0, 5'-H), 8.31 (1 H, dd, 1.8 and 8.0, 4'-H) and 8.75 (1 H, dd, 1.8 and 4.8, 6'-H); δ_C(CDCl₃) 18.04 (q, C-14), 18.78 (q, C-10'), 20.56 (q, 1-OCO*Me*), 20.56 (q, 8-OCO*Me*), 21.07 (q, 2-OCO*Me*), 21.07 (q, 7-OCO*Me*), 21.36 (q, 11-OCO*Me*), 21.64 (q, 5-OCO*Me*), 22.81 (q, C-12), 33.34 (t, C-8'), 33.52 (t, C-7'), 38.56 (d, C-9'), 51.26 (d, C-6), 52.28 (s, C-9), 60.29 (t, C-11), 69.48 (d, C-2), 69.94 (s, C-4), 70.35 (t, C-15), 71.11 (d, C-8), 73.63 (d, C-1), 73.88 (d, C-5), 76.04 (d, C-3), 84.50 (s, C-13), 93.85 (s, C-10), 121.20 (d, C-5'), 124.40 (s, C-3'), 138.75 (d, C-4'), 153.26 (d, C-6'), 164.16 (s, C-2'), 166.95 (s, C-12'), 168.81 (s, 2-OCO), 169.04 (s, 8-OCO), 169.40 (s, 1-OCO), 169.92 (s, 5-OCO), 170.16 (s, 7-OCO), 170.26 (s, 11-OCO) and 175.27 (s, C-11').

Ebenifoline E-I **5**.—Amorphous solid, m.p. 183–185 °C; [α]$_D$ 0 (*c* 0.71, CHCl$_3$); λ$_{max}$(EtOH)/nm (Δε) 246 (+3.3) and 227 (−3.8); *m/z* 825 (M$^+$), 766, 590, 572, 262, 206, 178, 161, 107 and 105 (Found: M$^+$, 825.2800. C$_{41}$H$_{47}$NO$_{17}$ requires *M*, 825.2749); ν$_{max}$(CHCl$_3$)/cm^{-1} 3611, 3501 and 1741 m; λ$_{max}$(EtOH)/nm (log ε) 201 (4.57), 229 (4.40) and 264 (3.73); δ$_H$(CDCl$_3$) see Table 1; δ$_C$(CDCl$_3$) see Table 2.

Ebenifoline E-II **6**.—Amorphous solid, m.p. 174–177 °C; [α]$_D$ − 13.2 (*c* 2.77, CHCl$_3$); λ$_{max}$(EtOH)/nm (Δε) 250 (+6.6) and 236 (−7.5); *m/z* 929 (M$^+$), 634, 262, 206, 178, 161, 107 and 105 (Found: M$^+$, 929.3086. C$_{48}$H$_{51}$NO$_{18}$ requires *M*, 929.3066); ν$_{max}$(CHCl$_3$)/cm^{-1} 3501 and 1747 m; λ$_{max}$(EtOH)/nm (log ε) 201 (4.66), 231 (4.60) and 266 (3.77); δ$_H$(CDCl$_3$) see Table 1; δ$_C$(CDCl$_3$) see Table 2.

Ebenifoline E-III **7**.—Amorphous solid, m.p. 174–177 °C; [α]$_D$ − 25.8 (*c* 0.67, CHCl$_3$); λ$_{max}$(EtOH)/nm (Δε) 240 (−12.1) and 221 (+7.2); *m/z* 929 (M$^+$), 870, 634, 572, 206, 178, 161 and 105 (Found: [M + H]$^+$, 930.3210. C$_{48}$H$_{52}$NO$_{18}$ requires MH, 930.3184); ν$_{max}$(CHCl$_3$)/cm^{-1} 3486 and 1752 m; λ$_{max}$(EtOH)/nm (log ε) 202 (4.49), 229 (4.44) and 265 (3.65); δ$_H$(CDCl$_3$) see Table 1; δ$_C$(CDCl$_3$) see Table 2.

Ebenifoline E-IV **8**.—Amorphous solid, m.p. 143–146 °C; [α]$_D$ − 7.8 (*c* 0.25, CHCl$_3$); λ$_{max}$(EtOH)/nm (Δε) 244 (+6.6) and 226 (−3.5); *m/z* 851 (M$^+$), 792, 748, 206, 178, 161 and 105 (Found: [M + H]$^+$, 852.3109. C$_{43}$H$_{50}$NO$_{17}$ requires MH, 852.3079); ν$_{max}$(CHCl$_3$)/cm^{-1} 1741 m; λ$_{max}$(EtOH)/nm (log ε) 201 (4.38), 229 (4.22) and 264 (3.57); δ$_H$(CDCl$_3$) see Table 1; δ$_C$(CDCl$_3$) see Table 2.

Ebenifoline E-V **9**.—Amorphous solid, m.p. 165–169 °C; [α]$_D$ − 27.5 (*c* 0.27, CHCl$_3$); λ$_{max}$(EtOH)/nm (Δε) 241 (−7.9) and 222 (+11.2); *m/z* 887 (M$^+$), 530, 262, 220, 206, 178, 161 and 105 (Found: [M + H]$^+$, 888.3051. C$_{46}$H$_{50}$NO$_{17}$ requires MH, 888.3079); ν$_{max}$(CHCl$_3$)/cm^{-1} 3392 and 1740 m; λ$_{max}$(EtOH)/nm (log ε) 202 (4.50), 229 (4.42) and 264 (3.63); δ$_H$(CDCl$_3$) see Table 1; δ$_C$(CDCl$_3$) see Table 2.

Euojaponine C **10**.—Amorphous solid, m.p. 178–182 °C; [α]$_D$ − 1.1 (*c* 0.12, CHCl$_3$); *m/z* 887 (M$^+$), 766, 652, 322, 262, 206 and 105; ν$_{max}$(CHCl$_3$)/cm^{-1} 3610, 3516 and 1724 m; λ$_{max}$(EtOH)/nm (log ε) 201 (4.58), 231 (4.57), 265 (3.75), 268 (3.74) and 280 (348); δ$_H$(CDCl$_3$) 1.18 (3 H, d, 7.1, 10′-H$_3$), 1.43 (3 H, s, 8-OAc), 1.43 (3 H, d, 7.0, 9′-H$_3$), 1.64 (3 H, d, 1.2, 12-H$_3$),1.73 (3 H, s, 14-H$_3$), 2.19 (3 H, s, 7-OAc), 2.35 (3 H, s, 11-OAc), 2.53 (1 H, d, 4.1, 6-H), 2.60 (1 H, q, 7.1, 8′-H), 2.65 (1 H, br br s, 2-OH), 3.61 (1 H, d, 11.5, 15-H^A), 4.18 (1 H, br s, 2-H), 4.73 (1 H, d, 1.2, 4-OH), 4.73 (1 H, q, 7.0, 7′-H), 4.87 (1 H, d, 2.5, 3-H), 4.91 (1 H, d, 13.6, 11-H^A), 5.46 (1 H, d, 5.8, 8-H), 5.52 (1 H, d, 13.6, 11-H^B), 5.58 (1 H, dd, 4.1 and 5.8, 7-H), 5.81 (1 H, d, 3.9, 1-H), 6.05 (1 H, d, 11.5, 15-H^B), 7.21 (1 H, s, 5-H), 7.26 (1 H, dd, 4.9 and 7.9, 5′-H), 7.43 (2 H, t-like, 7.7, 1-OBz *m*-H), 7.50 (2 H, t-like, 7.6, 5-OBz *m*-H), 7.56 (1 H, tt-like, 1.2 and 7.5, 1-OBz *p*-H), 7.59 (1 H, tt-like, 1.3 and 7.5, 5-OBz *p*-H), 7.97 (2 H, dd, 1.3 and 7.8, 1-OBz *o*-H), 8.04 (1 H, dd, 1.8 and 7.9, 4′-H), 8.35 (2 H, dd, 1.4 and 7.9, 5 OBz *o*-H and 8.70 (1 H, dd, 1.8 and 4.9, 6′-H); δ$_C$(CDCl$_3$) 9.44 (q, C-10′), 12.02 (q, C-9′), 18.42 (q, C-14), 20.05 (q, 8-OCO*Me*), 21.12 (q, 7-OCO*Me*), 21.64 (q, 11-OCO*Me*), 23.16 (q, C-12), 36.53 (d, C-7′), 45.18 (d, C-8′), 50.44 (d, C-6), 53.13 (s, C-9), 60.53 (t, C-11), 69.23 (d, C-7), 70.24 (d, C-2), 70.24 (t, C-15), 70.76 (s, C-4), 71.95 (d, C-8), 75.11 (d, C-5), 75.45 (d, C-1), 78.40 (d, C-3), 84.29 (s, C-13), 94.32 (s, C-10), 121.17 (d, C-5′), 125.54 (s, C-3′), 128.69 (2d, 1-OBz C-*m*), 128.88 (2d, 5-OBz C-*m*), 129.34 (s, 1-OBz C-*i*), 129.83 (s, 5-OBz C-*i*), 129.83 (2d, 1-OBz C-*o*), 130.44 (2d, 5-OBz C-*o*), 133.56 (d, 1-OBz C-*p*), 133.72 (d, 5-OBz C-*p*), 137.63 (d, C-4′), 151.48 (d,

C-6′), 164.99 (s, 1-OCO), 165.18 (s, C-2′), 165.91 (s, 5-OCO), 168.67 (s, C-12′), 169.13 (s, 8-OCO), 170.17 (s, 11-OCO), 170.24 (s, 7-OCO) and 174.56 (s, C-11′).

Mayteine **11**.—Amorphous solid, m.p. 168–170 °C; [α]$_D$ − 22.4 (*c* 0.18, CHCl$_3$); *m/z* 867 (M$^+$), 809, 572, 262, 206, 178, 161, 107 and 105; ν$_{max}$(CHCl$_3$)/cm^{-1} 3476 and 1741 m; λ$_{max}$(EtOH)/nm (log ε) 201 (4.28), 229 (4.09) and 265 (3.42); δ$_H$(CDCl$_3$) 1.23 (3 H, d, 7.1, 10′-H$_3$), 1.40 (3 H, s, 8-OAc), 1.41 (3 H, d, 7.0, 9′-H$_3$), 1.59 (3 H, d, 1.1, 12-H$_3$), 1.72 (3 H, s, 14-H$_3$), 2.12 (3 H, s, 7-OAc), 2.16 (3 H, s, 2-OAc), 2.22 (3 H, s, 5-OAc), 2.34 (3 H, s, 11-OAc), 2.36 (1 H, d, 4.1, 6-H), 2.62 (1 H, q, 7.1, 8′-H), 3.72 (1 H, d,11.3, 15-H^A) 4.51 (1 H, d, 1.1, 4-OH), 4.66 (1 H, q, 7.0, 7′-H), 4.66 (1 H, d, 13.5, 11-H^A), 4.80 (1 H, d, 2.4, 3-H), 5.35 (1 H, d, 13.5, 11-H^B), 5.35 (1 H dd, 2.4 and 4.1, 2-H), 5.42 (1 H, d, 5.8, 8-H), 5.53 (1 H, dd, 4.1 and 5.8, 7-H), 5.89 (1 H, d, 4.1, 1-H), 5.96 (1 H, d, 11.3, 15-H^B), 7.05 (1 H, s, 5-H), 7.27 (1 H, dd, 4.8 and 7.8, 5′-H), 7.39 (2 H, t-like, 7.8, 1-OBz *m*-H), 7.54 (1 H, tt-like, 1.2 and 7.5, 1-OBz *p*-H), 7.81 (2 H, dd, 1.4 and 8.4, 1-OBz *o*-H), 8.08 (1 H, dd, 1.8 and 7.8, 4′-H) and 8.71 (1 H, dd, 1.8 and 4.8, 6′-H); δ$_C$(CDCl$_3$) 9.90 (q, C-10′), 12.06 (q, C-9′), 18.53 (q, C-14), 19.96 (q, 8-OCO*Me*), 20.95 (q, 7-OCO*Me*), 21.04 (q, 2-OCO*Me*), 21.46 (q, 11-OCO*Me*), 21.72 (q, 5-OCO*Me*), 23.00 (q, C-12), 36.66 (d, C-7′), 45.04 (d, C-8′), 50.58 (d, C-6), 52.69 (s, C-9), 60.16 (t, C-11), 69.05 (d, C-7), 69.32 (d, C-2), 70.07 (t, C-15), 70.70 (s, C-4), 71.50 (d, C-8), 73.60 (d, C-1), 73.97 (d, C-5), 75.78 (d, C-3), 84.47 (s, C-13), 94.21 (s, C-10), 121.19 (d, C-5′), 125.19 (s, C-3′), 128.60 (2d, 1-OBz C-*m*), 129.29 (s, 1-OBz C-*i*), 129.61 (2d, 1-OBz C-*o*), 133.55 (d, 1-OBz C-*p*), 137.84 (d, C-4′), 151.62 (d, C-6′), 164.64 (s, C-2′), 165.46 (s, 1-OCO), 168.31 (s, 2-OCO), 168.60 (s, C-12′), 168.96 (s, 8-OCO), 169.96 (s, 5-OCO), 170.02 (s, 7-OCO), 170.44 (s, 11-OCO) and 174.09 (s, C-11′).

Euonymine **12**.—Amorphous solid, m.p. 140–144 °C; [α]$_D$ − 21.8 (*c* 0.22, CHCl$_3$); *m/z* 806 (M$^+$), 746, 686, 572, 262, 206, 178, 161 and 107; ν$_{max}$(CHCl$_3$)/cm^{-1} 3483 and 1746 m; λ$_{max}$(EtOH)/nm (log ε) 201 (3.92), 223 (3.61) and 265 (3.24); δ$_H$(CDCl$_3$) 1.19 (3 H, d, 7.1, 10′-H$_3$), 1.39 (3 H, d, 7.0, 9′-H$_3$), 1.54 (3 H, d, 1.1, 12-H$_3$), 1.67 (3 H, s, 14-H$_3$), 1.83 (3 H, s, 1-OAc), 1.99 (3 H, s, 8-OAc), 2.15 (3 H, s, 7-OAc), 2.15 (3 H, s, 2-OAc), 2.20 (3 H, s, 5-OAc), 2.31 (3 H, s, 11-OAc), 2.33 (1 H, d, 3.9, 6-H), 2.57 (1 H, q, 7.1, 8′-H), 3.69 (1 H, d, 11.5, 15-H^A), 4.48 (1 H, d, 1.1, 4-OH), 4.64 (1 H, q, 7.0, 7′-H), 4.72 (1 H, d, 2.5, 3-H), 5.13 (1 H, d, 13.5, 11-H^B), 5.23 (1 H, dd, 2.5 and 3.9, 2-H), 5.35 (1 H, d, 6.0, 8-H), 5.51 (1 H, dd, 3.9 and 6.0, 7-H), 5.55 (1 H, d, 3.9, 1-H), 5.94 (1 H, d, 11.5, 15-H^B), 7.02 (1 H, s, 5-H), 7.26 (1 H, dd, 4.8 and 7.8, 5′-H), 8.06 (1 H, dd, 1.8 and 7.8, 4′-H) and 8.69 (1 H, dd, 1.8 and 4.8, 6′-H); δ$_C$(CDCl$_3$) 9.79 (q, C-10′), 12.01 (q, C-9′), 18.58 (q, C-14), 20.50 (q, 1-OCO*Me*), 20.50 (q, 8-OCO*Me*), 21.08 (q, 2-OCO*Me*), 21.08 (q, 7-OCO*Me*), 21.40 (q, 11-OCO*Me*), 21.70 (q, 5-OCO*Me*), 23.02 (q, C-12), 36.59 (d, C-7′), 45.02 (d, C-8′), 50.65 (d, C-6), 52.32 (s, C-9), 60.11 (t, C-11), 68.94 (d, C-2), 69.12 (d, C-7), 69.99 (t, C-15), 70.66 (s, C-4), 70.83 (d, C-8), 73.43 (d, C-1), 73.91 (d, C-5), 75.85 (d, C-3), 84.25 (s, C-13), 94.15 (s, C-10), 121.17 (d, C-5′), 125.19 (s, C-3′), 137.81 (d, C-4′), 151.59 (d, C-6′), 165.42 (s, C-2′), 168.56 (s, C-12′), 168.67 (s, 2-OCO), 169.00 (s, 8-OCO), 169.13 (s, 1-OCO), 169.93 (s, 5-OCO), 170.10 (s, 7-OCO), 170.31 (s, 11-OCO) and 174.07 (s, C-11′).

Crystallographic Analysis of Compound **1**.—*Crystal data*: Chemical formula C$_{48}$H$_{51}$NO$_{18}$·H$_2$O, M_w = 947.9, crystal system monoclinic, space group; $P2_1$, Z = 2, a = 15.274(1), b = 15.716(3), c = 10.378(1) Å, V = 2430 Å^3, D_x = 1.295 g cm^{-3}, $F(000)$ = 1000. A single plate crystal of approximately 0.55 × 0.30 × 0.10 mm in size was mounted on a Nonius CAD4 diffractometer with graphite-monochromated Cu-Kα

radiation ($\mu = 8.1$ cm^{-1}) at 23 °C. A total of 4911 reflections were observed above the $2\sigma(I)$ level, with 2θ 6–150°. The structure was determined by direct methods using the SHELXS-86 program,[23] and refinement was carried out by block-diagonal-matrix least-squares. The final R-value was 0.056 $\{R_w = \Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2 = 0.003$, where, $\sqrt{w} = 0.1$ when $|F_o| \leqslant 1.0$, $\sqrt{w} = 1$ when $1.0 < |F_o| \leqslant 50$, $\sqrt{w} = 50/|F_o|$ when $|F_o| > 50$, $w = 0.1$ when $|F_o| < 1.0$ when $|F_o| > 50\}$. The number of atoms refined was 68 carbon, nitrogen and oxygen atoms with anisotropic thermal parameters, as well as 51 hydrogen atoms with isotropic parameters, which were found on the difference electron-density map and located at the calculated positions. Two hydrogen atoms of the solvate water molecule were not found. Final shift/esd-values were in the range 0.01–0.57 for non-hydrogen atoms. The maximum residual electron density was 0.41 e Å^{-3} [close to O(14)] with the average value 0.13 e Å^{-3}. The refined fractional atomic coordinates, bond distances and bond angles are available from the Cambridge Crystallographic Data Centre where they have been deposited.* The molecular structure determined by this method is illustrated in Fig. 3.

Acknowledgements

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* See Instructions for Authors, section 5.6.3, in the January issue.

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