g of benzoylated starting material; mp 103.5–104.5 °C; $[\alpha]^{24}_D$ −12.9° ($CHCl_3$) [lit.[26] $[\alpha]^{24}_D$ −12.8°]; IR (KBr) 1740 (ester), 1650 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.3 Hz), 1.26 (s, 22), 1.98–2.14 (m, 11; contains 3 s, δ 1.98, 2.06, 2.07, each ca. 3 H), 4.04 (dd, 1, J = 11.8, 3.7 Hz), 4.30 (dd, 1, J = 11.4, 5.8 Hz), 4.38–4.48 (m, 1), 5.28 (dd, 1, J = 7.35, 6.62 Hz), 5.39 (dd, 1, J = 15.4, 7.35 Hz), 5.68 (d, 1, J = 8.8 Hz), 5.79 (dt, 1, J = 15.1, 6.6 Hz).

Anal. Calcd for $C_{24}H_{43}NO_5$: C, 67.76; H, 10.12. Found: C, 67.66; H, 10.27.

The threo epimer of triacetylsphingosine was prepared in the same way, yield 84%, mp 43–43.5 °C, $[\alpha]^{24}_D$ +8.43° ($CHCl_3$); IR (KBr) 1740 (ester), 1660 (amide) cm^{-1}; NMR ($CDCl_3$) δ_H 0.88 (t, 3, J = 6.6 Hz), 1.25 (s, 22), 2.00–2.13 (m, 11; contains 3 s, δ 2.00, 2.07, 2.08, each ca. 3 H), 4.08 (m, 2), 4.35–4.45 (m, 1), 5.33–5.44 (m, 2), 5.67 (d, 1, J = 9.6 Hz), 5.78 (dt, 1, J = 14.7, 6.6 Hz). The multiplet at 4.08 ppm is the splitting pattern of the diastereotopic sphingosine C-1 protons which are coupled to the C-2 proton with J = 5.9 and 5.1 Hz and differ in chemical shift by 1.8 Hz.

Anal. Calcd for $C_{24}H_{43}NO_5$: C, 67.76; H, 10.12. Found: C, 67.99; H, 10.27.

Acknowledgment. Support of this work by the National Institutes of Health and assistance in the purchase of the NMR spectrometers by National Science Foundation Departmental Instrument grants are gratefully acknowledged. We also thank Dr. W. B. Wise for his technical assistance, Ms. D. Ward and Ms. T. Denial for their experimental assistance, and Ms. H. Brachowski for preparing the manuscript.

Registry No. 2, 78715-83-0; **3**, 78715-84-1; **4**, 41765-25-7; **5a**, 78715-85-2; **5b**, 78739-31-8; *erythro*-**6**, 78739-32-9; *threo*-**6**, 78739-33-0; **7a**, 78715-86-3; **7b**, 78779-90-5; **8a**, 5966-28-9; **8b**, 78779-91-6; **9a**, 78715-87-4; **9b**, 78779-92-7; **10a**, 78715-88-5; **10b**, 78779-93-8; **11a**, 78715-89-6; **11b**, 78779-94-9; **12a**, 73039-25-5; **12b**, 78779-95-0; L-serine, 56-45-1; L-serine methyl ester hydrochloride, 5680-80-8; *p*-nitrophenyl oleate, 17363-90-5; *tert*-butychlorodiphenylsilane, 58479-61-1; α-D-bromotetraacetylgalactose, 3068-32-4; *erythro*-*N*-acetyl-1-*O*-benzoylsphingosine, 78715-90-9; *threo*-*N*-acetyl-1-*O*-benzoylsphingosine, 78715-91-0; *erythro*-triacetylsphingosine, 2482-37-3; *threo*-triacetylsphingosine, 78779-96-1.

Supplementary Material Available: Carbon-13 NMR assignments for *erythro*- and *threo*-ceramides and galactosylceramides (3 pages). Ordering information is given on any current masthead page.

Novel Maytansinoid Tumor Inhibitors from *Trewia nudiflora*: Trewiasine, Dehydrotrewiasine, and Demethyltrewiasine[1]

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Alcoholic extracts of *Trewia nudiflora* seed have yielded several new maytansinoid tumor inhibitors that are exceptionally active in the PS, B1, and KB systems. These include trewiasine (**1**), dehydrotrewiasine (**2**), and demethyltrewiasine (**3**), which were characterized by NMR, mass spectral, and chemical correlation with other known maytansinoids. Two additional maytansinoids, trenudine (**10**) and treflorine (**11**), have been partially characterized. The *Trewia* ansa macrolides differ from others in this series in that they possess an additional methoxy group at C-15; and, with the exception of **1**, they contain substituents at C-3 which differ from other known maytansinoids. Detailed ^{13}C NMR assignments for maytansine and some related maytansinoids are presented.

In a search for tumor inhibitors of plant origin, we found that ethanolic extracts of *Trewia nudiflora* L. (Euphorbiaceae) seed[3] showed significant activity in vitro against human carcinoma of the nasopharynx (KB) and in vivo against P388 lymphocytic leukemia (PS).[4] *Trewia* extracts also inhibit initiation and growth of crown-gall tumors on potato disks.[5] Previous studies by other workers have demonstrated that *T. nudiflora* seed contains a highly unusual glyceride oil,[6] several novel pyridone alkaloids,[7-9] and an inhibitor of protein synthesis.[10] In this paper, we report the isolation and structural elucidation of three new maytansinoid tumor inhibitors: trewiasine (**1**), dehydrotrewiasine (**2**), and demethyltrewiasine (**3**). In addition, we describe the partial characterization of two more new maytansinoids, trenudine (**10**) and treflorine (**11**). Maytansinoids, including maytansine (**4**), colubrinol (**5**), and maytanbutine (**6**), have previously been reported as constituents of *Maytenus* and *Putterlickia* spp. (Celastraceae),[11] *Colubrina texensis* (Rhamnaceae),[12] and fermentation broths of a *Nocardia* sp.[13] These compounds are

(1) Presented in part at the 180th National Meeting of the American Chemical Society and the Second Chemical Congress of the North American Continent, Las Vegas, NV, Aug 24–29, 1980.

(2) The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

(3) We thank Dr. James Duke, USDA, Beltsville, MD, for supplying seed material in accordance with the program developed by the National Cancer Institute. A 27.2-kg recollection of *Trewia nudiflora* L. seed was purchased in 1978 from Pratap Nursery, Dehra Dun, India.

(4) Cytotoxic and antitumor activities were assayed under the auspices of the National Cancer Institute by the procedures described by: Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3*, **1972**, *3*, 1. Trewiasine (**1**) was cytotoxic at the 2.0×10^{-4} μg/mL level. In addition, **1** gave T/C values of 126–168 in the dosage range 1.0–31.0 μg/kg against PS and T/C values of 165–207 in the dosage range 4.0–32.0 μg/kg against B1.

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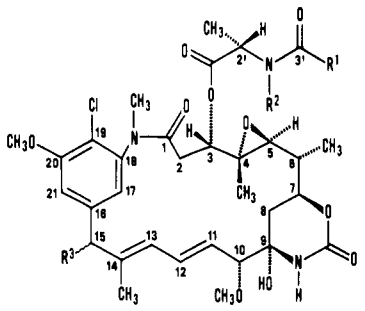
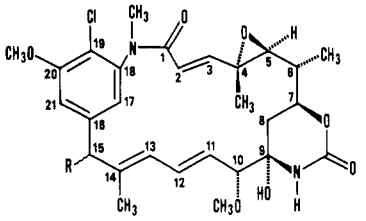
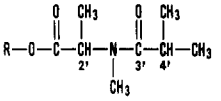


1 Trewiasine $R^1 = CH(CH_3)_2$; $R^2 = CH_3$; $R^3 = OCH_3$
2 Dehydrotrewiasine $R^1 = C(CH_3)=CH_2$; $R^2 = CH_3$; $R^3 = OCH_3$
3 Demethyltrewiasine $R^1 = CH(CH_3)_2$; $R^2 = H$; $R^3 = OCH_3$
4 Maytansine $R^1 = CH_3$; $R^2 = CH_3$; $R^3 = H$
5 Colubrinol $R^1 = CH(CH_3)_2$; $R^2 = CH_3$; $R^3 = OH$
6 Maytanbutine $R^1 = CH(CH_3)_2$; $R^2 = CH_3$; $R^3 = H$



7 Trewsine R = OCH_3
8 Maysine R = H



9a R = H
9b R = CH_3

of interest as antineoplastic agents, and maytansine has been the target of intensive synthetic efforts.[14,15] In a decision based on its availability, relative to other known maytansinoids, maytansine is currently undergoing extensive clinical trials.[16] In a different context, we find that crude *Trewia* extract and trewiasine are biologically active against various insects, suggesting possible use as a pest control agent. Results of these biological experiments with insects will be described elsewhere.[17]

Fractionation (Chart I) of the ethanolic extract of defatted *Trewia* seed was guided by assay against KB cell culture and PS leukemia in mice. Partition of the extract between chloroform and water gave concentration of activity in the chloroform layer. The chloroform-soluble fraction was then subjected to column chromatography on silica using a stepwise gradient of increasing concentrations of methanol in chloroform. The fraction of highest activity was rechromatographed on silica by preparative HPLC using a stepwise gradient of dichloromethane–methanol with increasing concentrations of methanol. Activity was then further enriched by preparative HPLC on a reversed-phase (C_{18}) column eluted with a stepwise gradient of increasing methanol in water or by HPLC on a smaller scale with methanol–water (70:30). Appropriate highly active fractions were combined on the basis of TLC, and the most abundant active compound, 1, was purified by repeated HPLC of fraction C on a C_{18} μ-Bondapak column with similar methanol–water mixtures. Fraction B, eluted immediately prior to 1, was then subjected to preparative TLC which yielded two additional active compounds (2 and 3).

Trewiasine (1) was obtained crystalline from dichloromethane (mp 182–185 °C) in an overall yield of 800 mg (3.3×10^{-3}%). Comparison of the ^1H NMR spectra of 1 and colubrinol (5)[18] indicated that these two compounds were closely related; the only significant differences were the presence of an additional methoxyl signal at δ 3.37 in the spectrum of 1 and an upfield shift of the C-15 proton singlet from δ 5.48 in 6 to δ 4.86 in 1. Further evidence favoring structure 1 was obtained by comparing the ^1H and ^{13}C NMR spectra of 1 and maytansine (4) (Tables I and II). The C-15 carbon signal of 4 (t, δ 46.6) was shifted downfield in 1 (d, δ 86.7), and the C-15 proton doublets of 4 (δ 3.10 and δ 3.63) were replaced by a downfield singlet at δ 4.86 in the spectrum of 1. Thus, 1 was determined to have the maytansinoid ring system with a methoxyl at C-15. A mass spectrum gave an apparent $M^+ - (H_2O + HNCO)$ at m/z 688.3090 (calcd 688.3126) confirming that 1 was $C_{37}H_{52}ClN_3O_{11}$ (mol wt 749). Compound 1 also gave a characteristic ion at m/z 515 ($C_{28}H_{34}ClNO_6$) corresponding to an additional loss of the C-3 substituent. Maytansinoid fragmentations of this type, M^+ - (a + b), are well documented.[11,13]

Hydrolysis of 1 with sodium carbonate in 50% aqueous methanol at room temperature gave trewsine (7), identified by spectral characteristics and by comparison with maysine (8).[11] The ^1H NMR spectrum of 7 differed from that of 8 only in having a proton singlet at δ 4.67 and an additional methoxyl singlet at δ 3.37, rather than the two doublets at δ 3.02 and 3.42 previously attributed to the C-15 protons of 8. In addition, the mass spectrum of 7 gave a characteristic ion at m/z 515, M^+ – a, which was 30 mass units (OCH_2) higher than the corresponding peak at m/z 485 in the spectrum of 8. The other hydrolysis product of 1 was characterized as *N*-isobutyryl-*N*-methylalanine (**9a**) by GC/MS of its methyl ester (**9b**). Thus, 1 was identified as 15-methoxymaytanbutine (15-*O*-methylcolubrinol).

The structure of dehydrotrewiasine (2) was readily deduced from spectral data. The M^+ – a peak in the mass spectrum appears at m/z 686, corresponding to mol wt 747 ($C_{37}H_{50}ClN_3O_{11}$, 2 amu less than 1); however, the M^+ – (a + b) peak remains at m/z 515. Four of the highest intensity ions in the mass spectrum of 2 (m/z 41, 69, 162, and 154) all arise from fragmentation of the C-3 ester and are all two units less than the corresponding peaks in the mass spectrum of 1. Another prominent ion, present at m/z 58 in the spectra of 1, 2, and **9b**, must arise by cleavage adjacent to both carbonyl groups in the central portion of the ester and abstraction of a proton to yield $[CH_3CH{=}NHCH_3]^+$. The ^1H NMR spectrum of 2 differs substantially from that of 1 in that the 4′-proton signal (δ 2.76) and the 4′-methyl doublets (δ 1.06 and 1.11) in the spectrum of 1 are replaced by a methyl singlet at δ 1.92 and two olefinic proton signals (δ 5.02 and 5.22). The ^{13}C NMR spectrum also supports these assignments since an additional double bond is documented by signals at δ 138.95 (a singlet) and δ 116.53 (a triplet); this double bond must have two protons attached at one carbon and none at the other. It is apparent that 2 differs from 1 only in that the former possesses a double bond at the terminal end of the C-3 ester side chain.

Demethyltrewiasine (3) gave an M^+ – a peak in the mass spectrum at m/z 674, corresponding to mol wt 735 ($C_{36}H_{50}ClN_3O_{11}$), and an M^+ – (a + b) peak at m/z 515. The spectrum of 3 also exhibited peaks arising from the C-3

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Table I. ^{1}H NMR Data of *Trewia nudiflora* Components and Related Maytansinoids[a]

proton assignments	compd 1	2	3	4	7
2_A	2.18 (dd, J = 14.3, 3.0)	2.18 (dd, J = 14.4, 2.9)	2.18 (dd, J = 14.0, 3.0)	2.16 (dd, J = 14.4, 3.1)	5.67 (d, J = 15.5)
2_B	2.55 (dd, J = 14.3, 12.2)	2.59 (dd, J = 14.4, 12.2)	2.55 (dd, J = 14.0, 12.0)	2.60 (dd, J = 14.4, 12.1)	
3	4.75 (dd, J = 12.2, 3.0)	4.86 (dd, J = 12.2, 2.9)	4.84 (dd, J = 12.0, 3.0)	4.75 (dd, J = 12.1, 3.1)	6.42 (d, J = 15.5)
4-CH_3	0.76 (s)	0.80 (s)	0.82 (s)	0.78 (s)	1.04 (s)
5	3.01 (d, J = 9.6)	3.00 (d, J = 9.5)	2.88 (d, J = 9.5)	3.00 (d, J = 9.6)	2.66 (d, J = 9.8)
6-CH_3	1.27 (d, J = 6.2)	1.28 (d, J = 6.3)	1.28 (d, J = 6.0)	1.27 (d, J = 6.4)	1.31 (d, J = 6.4)
7	4.28 (m)	4.26 (m)	4.22 (m)	4.26 (m)	4.30 (m)
10	3.51 (d, J = 9.1)	3.52 (d, J = 9.1)	3.52 (d, J = 9.0)	3.47 (d, J = 9.1)	3.46 (d, J = 9.4)
11	5.72 (dd, J = 15.3, 9.1)	5.81 (dd, J = 15.3, 9.1)	5.73 (dd, J= 15.0, 9.0)	5.65 (dd, J = 15.3, 9.1)	5.58 (dd, J = 15.3, 9.4)
12	6.46 (dd, J = 15.3, 11.1)	6.47 (dd, J = 15.3, 11.2)	6.46 (dd, J = 15.0, 11.0)	6.41 (dd, J = 15.3, 11.1)	6.44 (dd, J = 15.3, 10.9)
13	6.98 (d, J = 11.1)	6.90 (d, J = 11.2)	6.90 (d, J = 11.0)	6.65 (d, J = 11.1)	6.24 (d, J = 10.9)
14-CH_3	1.52 (s)	1.52 (s)	1.55 (s)	1.62 (s)	1.56 (s)
15_A	4.86 (s)	4.78 (s)	4.84 (s)	3.10 (d, J = 12.4)	4.67 (s)
15_B				3.63 (d, J = 12.4)	
17	6.54 (d, J = 1.5)	6.64 (d, J = 1.5)	6.68 (d, J = 1.5)	6.72 (d, J = 1.7)	6.60 (d, J = 1.6)
21	7.22 (d, J = 1.5)	7.23 (d, J = 1.5)	7.23 (d, J = 1.5)	6.81 (d, J = 1.7)	7.20 (d, J = 1.6)
10-OCH_3[b]	3.35 (s)	3.35 (s)	3.38 (s)	3.33 (s)	3.34 (s)
15-OCH_3[b]	3.37 (s)	3.36 (s)	3.38 (s)		3.37 (s)
20-OCH_3	3.99 (s)	3.99 (s)	4.00 (s)	3.96 (s)	4.00 (s)
18-NCH_3	3.16 (s)	3.14 (s)	3.14 (s)	3.18 (s)	3.25 (s)
2′	5.37 (m)	5.29 (m)	4.90 (m)	5.32 (q)	
2′-CH_3	1.28 (d, J = 6.8)	1.33 (d, J = 6.9)	1.35 (d, J = 7.0)	1.29 (d, J = 6.9)	
2′-NCH_3	2.88 (s)	2.88 (s)		2.84 (s)	
4′	2.76 (m)		2.30 (m)	2.09 (s)	
4′-CH_3	1.06 (d, J = 6.6)	1.92 (s)	1.08 (d, J = 7.0)		
4′-CH_3	1.12 (d, J = 6.8)		1.16 (d, J = 7.0)		
4′-CH_2		5.02 (s), 5.22 (s)			

[a] Chemical shifts (δ) are expressed in parts per million from tetramethylsilane, and coupling constants (J) are expressed in hertz. Extensive decoupling was used to verify assignments. Spectra were recorded in deuteriochloroform solution on a Fourier transform Bruker WH-90 spectrometer and, with the exception of **3**, also on a Nicolet NT-360 spectrometer. In all of the above compounds the C-6 proton signal occurs at ca. δ 8.13 but is obscured by other signals; the 9-NH proton is observed as a broad singlet in the δ 6.2–6.4 region. [b] These assignments may be reversed.

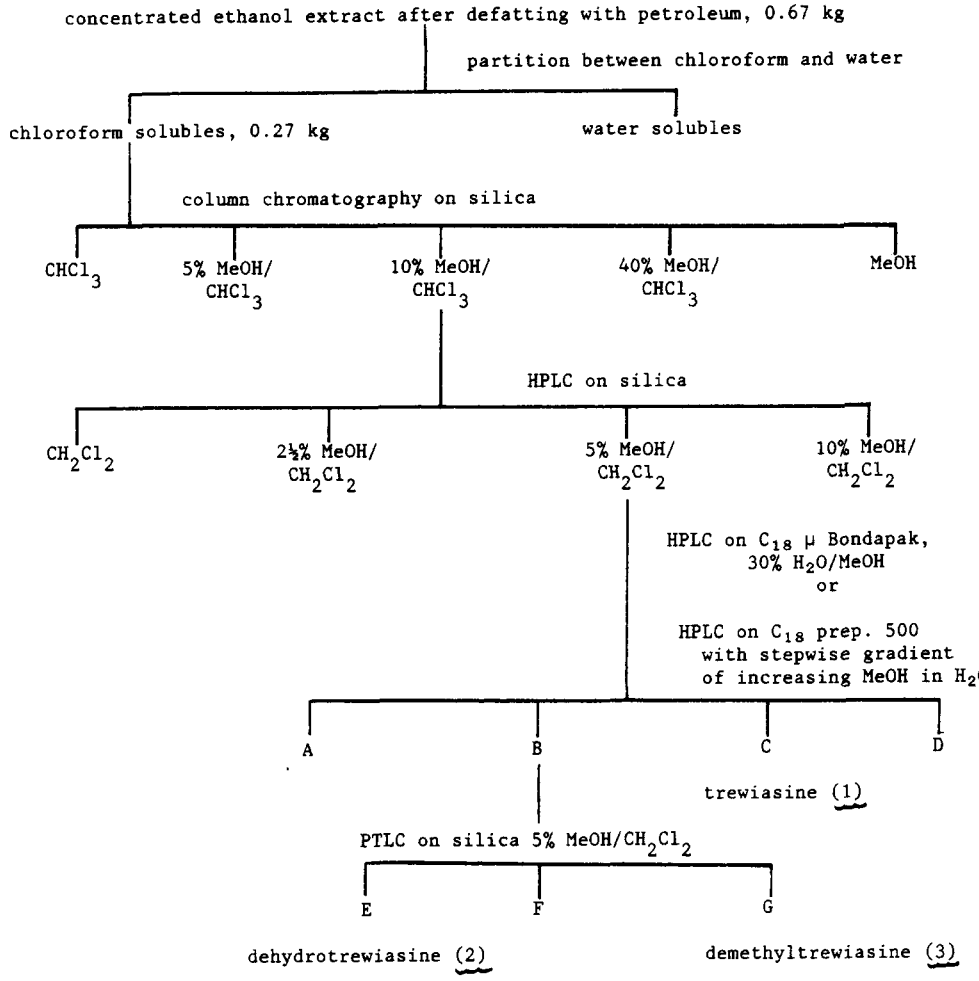
Table II. ^{13}C NMR Data of *Trewia nudiflora* Components and Related Maytansinoids[a]

carbon assignments	compd 1	2	4	7	carbon assignments	compd 1	2	4	7
2	32.43 t	32.43 t	32.37 t	119.71 d	C=O	176.71 s	173.33 s	170.86 s	
3	78.18 d	77.92 d	78.12 d	147.59 d	C=O	170.92 s	171.96 s	170.34 s	
4	59.99 s	60.12 s	60.06 s	59.53 s	C=O	168.84 s	170.47 s	168.78 s	164.23 s
5	67.73 d	66.87 d	67.20 d	66.68 d	C=O	152.40 s	152.27 s	152.34 s	152.21 s
6	38.86 d	38.86 d	38.86 d	38.54 d	OCH_3	56.3-7 3q	56.2-8 3q	56.61 2q	56.61 3q
7	74.15 d	74.09 d	74.09 d	74.80 d	CH_3	14.62 q	14.56 q	15.47 q	14.82 q
8	36.26 t	36.13 t	36.20 t	35.16 t	CH_3	13.13 q	13.26 q	14.56 q	14.10 q
9	80.72 s	80.78 s	80.59 s	80.91 s	CH_3	11.96 q	12.22 q	13.32 q	
10	85.52 d	88.39 d	88.58 d	88.26 d	CH_3	10.01 q	9.94 q	12.09 q	9.88 q
11	129.92 d	129.98 d	127.79 d	129.01 d	18-NCH_3	35.22 q	35.42 q	35.42 q	35.87 q
12	132.51 d	132.51 d	133.23 d	132.06 d	2′-NCH_3	30.42 q	32.43 q	31.78 q	
13	127.97 d	127.97 d	125.30 d	126.08 d	2′	52.38 d	52.38 d	52.25 d	
14	142.13 s	142.00 s	142.13 s	141.42 s	4′	30.42 d	138.95 s	21.90 q	
15	86.70 d	86.83 d	46.60 t	87.22 d	4′-CH_3	19.43 q	20.21 q		
16	141.35 s	141.48 s	141.16 s	141.09	4′-CH_3	18.85 q			
17	120.30 d	120.10 d	122.18 d	121.66 d	4′-CH_2		116.53 t		
18	139.01 s	139.99 s	139.21	140.64 s					
19	118.93 s	119.91 s	118.74 s	*b*					
20	156.30 s	156.37 s	155.91 s	156.50 s					
21	108.96 d	108.79 d	113.15 d	108.01 d					

[a] Chemical shifts (δ) are expressed in parts per million from tetramethylsilane. Proton-decoupled and off-resonance-decoupled spectra were recorded in deuteriochloroform solution on a Fourier transform Bruker WH-90 spectrometer. [b] Not observed.

ester at m/z 43, 71, 114, and 142, and the base peak appears at m/z 44, corresponding to $[CH_3CH{=}NH_2]^+$. The ^{1}H NMR spectrum of **3** was almost identical with that of **1** except that the former lacked one singlet, at δ 2.88, due to an *N*-methyl group while retaining the other *N*-methyl singlet at δ 3.14. We conclude that the side-chain *N*-

Chart I. Fractionation of the Active Extract from 27.2 kg of *Trewia nudiflora* Seed



methyl group is the one absent in **3** as this signal is also absent in the ansamitocins which have no nitrogen in their ester side chains.[13] In contrast, the side-chain *N*-methyl signal of normaytansine appears at δ 2.85.[19] These data demonstrate that **3** can only be the 2′ *N*-demethyl analogue of **1**.

The structures of **1**, **2**, and **7** were further documented by detailed analysis of their ^{13}C NMR spectra (Table II). To our knowledge, complete assignment of ^{13}C NMR signals in the maytansinoid series has not been published previously. With maytansine as our model, we compared the ^{13}C chemical shifts of the compounds isolated in our laboratory from *T. nudiflora*. There are three obvious peaks (δ 164–176) in the ester or amide carbonyl region of all these compounds. The molecular structures require a fourth carbonyl, and we believe this absorption, probably due to the carbonyl between oxygen and nitrogen of the carbinol amide grouping, appears at δ 152.4 ± 0.2. This structure is somewhat analogous to 1,3-dimethyluracil where the carbonyl signal is found at δ 151.8[20] Trewsine (**7**) lacks the ester side chain and exhibits only two carbonyl signals (δ 164.2 and 152.2).

Assignments for the aromatic carbons can be calculated from published[21] substituent constants, and the consistent set of values shown in Table II is obtained. In particular, the chlorine-bearing carbon, C-19, has a long relaxation time and is readily assigned due to its sharpness and reduced amplitude.[21] The olefinic carbons are assigned by taking into account their substituents and expected multiplicities. There are three methylene groups in maytansine (C-2, C-8, C-15). We have assigned the resonance at δ 46.6 to C-15 on the basis of the expected downfield shift observed when C-15 is oxygenated in **1**, **2**, and **7**. The resonance at δ 32.4 is absent in trewsine and is, therefore, assigned to C-2. The remaining methylene (C-8), which is expected to undergo the least change, is found near δ 36.2 in all four compounds.

Epoxy-bearing carbons C-4 and C-5 can be assigned by their shifts and multiplicities. The oxygenated methine carbons are C-3, C-7, C-10, and (except for maytansine) C-15. In trewsine, C-3 becomes an olefinic carbon; and since the δ 78.1 resonance is absent in trewsine, it is assigned to C-3. C-15 is assigned to the peak at δ 86.7 because it is not found in maytansine. The remaining oxygenated carbon doublets at δ 74.1 and 88.5 are assigned to C-7 and C-10, respectively, from their chemical shifts. Maytansine contains two methylated methine carbons at C-6 and C-2′ which are distinguished by their shifts and the absence of a peak for C-2′ in the hydrolysis–elimination product, trewsine.

Other maytansinoids are present in *T. nudiflora*, including two unique compounds, trenudine (**10**) and treflorine (**11**), which have been partially characterized. Trenudine (mp 200–205 °C dec, $C_{36}H_{48}ClN_3O_{13}$) occurs in fraction A and treflorine (mp 205–208 °C dec) was isolated from fraction F. ^1H and ^{13}C NMR spectra of **10** and **11**

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confirm the maytansinoid ring system of 1; however, no signals attributable to *N*-methyl groups are observed. Both compounds fail to give $M^+ - (a + b)$ ions characteristic of other maytansinoid esters. Calculations based on the molecular weight, as determined by high-resolution mass spectroscopy, indicate that both contain an additional ring or double bond equivalent. These observations lead us to postulate that both **10** and **11** contain an additional macrocyclic ring linking C-3 and the aromatic amide nitrogen. Limited amounts of these materials available for chemical studies and difficulties encountered in our interpretation of available spectral data preclude full disclosure of structures for **10** and **11** at this time. Further work is in progress.

Experimental Section

General Procedures. Melting points were determined with a Fischer-Johns block and are uncorrected. Analytical and preparative TLC was accomplished with silica gel 60 F-254 plates (0.25 mm thick, E. Merck). Plates were developed with CH_2Cl_2–MeOH (95:5) and visualized under UV light. Hi Flosil (60/200 mesh, Applied Science) was used for column chromatography. HPLC was performed with a Waters Associates, Inc., Preparative 500 LC system (using silica of C_{18} bonded silica cartridges, as appropriate) or with a Model ALC/PC-201 instrument equipped with a 7.8 × 300 mm, C_{18}, μ-Bondapak column and an RI detector, and operated at a flow rate of 2 mL/min. IR spectra were recorded on a Perkin-Elmer Model 700 instrument with 1% $CHCl_3$ solutions, and optical rotations were determined with a Perkin-Elmer Model 241 polarimeter. ^1H (90 MHz) and ^{13}C NMR (22.63 MHz) spectra were determined with a Bruker WH-90 instrument; $CDCl_3$ solutions were used with tetramethylsilane as an internal standard. High-field ^1H spectra (360 MHz) were recorded on a Nicolet NT-360 instrument, and extensive decoupling was used to verify assignments. Low-resolution mass spectra were obtained with a Du Pont (CEC) 21-492-1 spectrometer and high-resolution mass spectra with either a Nuclide 12-90-DF or Kratos MS-30 instrument.

Extraction Procedures. *Trewia nudiflora* seed (27.2 kg) was ground in a Wiley mill, divided into four batches, and defatted with hexane in a pilot-plant-scale Soxhlet extractor, yielding 4.07 kg of oil. Each batch was then further extracted with 95% ethanol to provide a combined total of 668 g of ethanol-soluble material. Ethanol solubles were then partitioned between $CHCl_3$ and H_2O (Chart I) in eight portions of approximately 83 g each. Each portion was then partitioned between 1 L of H_2O and 0.75 L of $CHCl_3$ followed by washing of the H_2O layer three times with 0.5 L of $CHCl_3$. Evaporation to dryness gave 283.3 g of $CHCl_3$-soluble material.

Chromatographic Separations. A 274-g portion of $CHCl_3$-soluble material was divided into nine portions of approximately 30 g each and subjected to column chromatography on columns packed with 270 g of silica. The eluting solvents for each of the nine runs consisted of a step-wise gradient of methanol in chloroform: 1.5 L of $CHCl_3$, 1.5 L of $CHCl_3$–MeOH (95:5), 1.5 L of $CHCl_3$–MeOH (90:10), 1.5 L of $CHCl_3$–MeOH (60:40), and 1.5 L of MeOH. Similar fractions were combined on the basis of TLC analysis, and the most active material (32 g) was collected near the middle of the run or during elution with $CHCl_3$–MeOH (90:10).

Further separation was achieved by preparative HPLC of the most active fraction, 30.4 g, in three runs of approximately 10.1 g each by using a stepwise gradient of increasing methanol in dichloromethane. Eluting solvents for each run included 100 mL of CH_2Cl_2, 1.5 L of CH_2Cl_2–MeOH (97.5:2.5), 1.5 L of CH_2Cl_2–MeOH (95:5), and 2.0 L of CH_2Cl_2–MeOH (90:10). Similar fractions were again combined on the basis of TLC analysis. The fraction of highest activity (5.3 g) was collected during elution with CH_2Cl_2–MeOH (95:5). This material was further separated into four characteristic fractions (A–D) by either of two methods (I and II). In method I, a 1.6-g portion was subjected to semipreparative HPLC on a C_{18} μ-Bondapak column by using a solvent consisting of MeOH–H_2O (70:30; 107 injections of approximately 15 mg/injection); this procedure yielded 289 mg of A, 72 mg of B, 195 mg of C and 833 mg of D. By use of method II, the remaining highly active material, 3.7 g, was deposited on approximately 90 cm^3 of C_{18} silica packed in a precolumn, which was placed in the system ahead of a C_{18} preparative 500 cartridge. The column was then eluted with the following: 4.5 L of MeOH–H_2O (60:40), which yielded A, 450 mg; MeOH (1 L), which gave 2.1 g of a mixture, mainly B and C; 1 L of CH_2Cl_2, which gave 1.1 g of D. The 2.1-g mixture of B and C was then rechromatographed under similar conditions except that the column was eluted with MeOH–H_2O (70:30, 2 L) and MeOH–H_2O (80:20, 2.5 L) and then washed with MeOH followed by CH_2Cl_2. An additional 62 mg of B and 782 mg of C was obtained.

Trewiasine (1). Fraction C was subjected to a final HPLC separation on C_{18} μ-Bondapak in 15-mg portions by using MeOH–H_2O (70:30). This procedure gave **1**: 800 mg (3.3×10^{-3}% yield based on seed material); mp 182–185 °C (after repeated recrystallization from CH_2Cl_2–hexane); IR ($CHCl_3$) 3600, 3450, 1750, 1715, 1665, 1640, 1590 cm^{-1}; UV_{max} (EtOH) 233 nm (ε 27 000), 243 (sh, 23 600), 254 (26 200), 282 (6700), 289 (6000); $[\alpha]^{23}_D$ −94° (*c* 0.15, $CHCl_3$); ^1H and ^{13}C NMR (see Tables I and II); mass spectrum (70 eV), *m/z* (relative intensity) 688 (M^+ − a, 1.6), 515 (3.3), 500 (1.4), 484 (1.5), 448 (1.9), 156 (55.4), 128 (16.9), 109 (12.2), 71 (11.6), 58 (100), 43 (35.5); found for $M^+ - (H_2O + HNCO)$ *m/z* 688.3090, $C_{36}H_{49}ClN_2O_9$ requires *m/z* 688.3126.

Conversion of Trewiasine to Trewsine (7) and Isolation of *N*-Isobutyryl-*N*-methylalanine Methyl Ester (9b). A mixture of **1** (64 mg) and sodium carbonate (45 mg) in 6 mL of 50% aqueous methanol was stirred at room temperature for 3.5 h. The reaction mixture was acidified with 10 mL of 2% aqueous tartaric acid solution and extracted with $CHCl_3$ which upon evaporation yielded 61 mg of yellow solid. This solid was purified by preparative TLC on silica developed with $CHCl_3$–MeOH (95:5) to yield (along with a number of unidentified products) trewsine (**7**): 11 mg; mp 147–154 °C; IR ($CHCl_3$) 3600, 3450, 1720, 1675, 1640, 1590 cm^{-1}; $[\alpha]^{23}_D$ −144° (*c* 0.055, $CHCl_3$)]; ^1H and ^{13}C NMR spectra of **7** are reported in Tables I and II; mass spectrum (70 eV), *m/z* (relative intensity) 515 (M^+ − a, 30.6), 480 (26.0), 324 (13.1), 214 (16), 191 (21.1), 111 (25.7), 95 (34.6), 83 (28.6), 75 (95.0), 58 (58.3), 55 (95.4), 53 (72.3), 44 (72.5), 43 (100); found for $M^+ - (H_2O + HNCO)$ *m/z* 515.2112, $C_{28}H_{34}ClNO_6$ requires *m/z* 515.2074.

The remaining hydrolysis products were treated with ethereal diazomethane and subjected to analysis by GC/MS for identification of **9b**: mass spectrum (70 eV), *m/z* (relative intensity) 187 (M^+, 2.6), 156 (1.0), 128 (31.0), 116 (10.0), 71 (9.0), 58 (100), 43 (44.6); found *m/z* 187.1222 (M^+), $C_9H_{17}NO_3$ requires *m/z* 187.1208.

Dehydrotrewiasine (2). Preparative TLC of fraction B on silica plates developed with CH_2Cl_2–MeOH (95:5) gave three major bands (E–G in order of decreasing R_f). Band E gave **2**: 30 mg (1.1×10^{-4}% yield); mp 165–170 °C after recrystallization from CH_2Cl_2–hexane; IR ($CHCl_3$) 3600, 3450, 1750, 1715, 1665, 1630, 1590 cm^{-1}; UV_{max} (EtOH) 233 nm (ε 23 600), 243 (sh, 19 700), 254 (21 000), 282 (5240), 289 (4870); $[\alpha]^{23}_D$ −90° (*c* 0.12, $CHCl_3$); ^1H and ^{13}C NMR (see Tables I and II); mass spectrum (70 eV), *m/z* (relative intensity) 686 (M^+ − a, 1.7), 515 (2.8), 500 (1.8), 484 (2.5), 448 (1.7), 154 (29.2), 126 (50.5), 69 (100), 58 (40.7) 41 (51.1).

Demethyltrewiasine (3). Band G, from preparative TLC of fraction B, gave substantially pure **3**: 15 mg (5.5×10^{-5}% yield); mp 129–142 °C (after recrystallization from CH_2Cl_2–hexane); IR ($CHCl_3$) 3600, 3450, 1750, 1715, 1675, 1590 cm^{-1}; UV_{max} (EtOH) 233 nm (ε 23 200), 243 (sh, 19 300), 254 (20 800), 282 (5100), 289 (5000); $[\alpha]^{23}_D$ −126° (*c* 0.049, $CHCl_3$); ^1H NMR (see Table I); mass spectrum (70 eV), *m/z* (relative intensity) 674 (M^+ − a, 2.3), 515 (8.7), 500 (5.5), 484 (4.5), 448 (5.5), 142 (20.3), 114 (25.3), 109 (32.8), 75 (19.4), 71 (27.2), 44 (100), 43 (85).

Trenudine (10). Fraction A was subjected to a final HPLC separation on C_{18} μ-Bondapak in 15-mg portions by using MeOH–H_2O (65:35). This gave **10**: 186 mg (6.9×10^{-4}% yield based on seed material); mp 200–205 °C dec (after recrystallization from CH_2Cl_2–hexane); IR ($CHCl_3$) 3600, 3450, 3360, 1760, 1715, 1665, 1610, 1590 cm^{-1}; UV_{max} (EtOH) 233 nm (ε 26 400), 248 (sh, 21 500), 253 (22 600), 282 (6130), 288 (6130); $[\alpha]^{23}_D$ −114° (*c* 0.24, $CHCl_3$); mass spectrum (70 eV), *m/z* (relative intensity) 704 (M^+ − a, 3.9) 672 (2.4), 669 (2.6), 593 (2.6), 204 (3.2), 185 (3.1), 171 (5.5), 95 (10.2), 85 (10.5), 83 (11.7), 81 (11.5), 69 (16.9), 55 (23.9), 44 (100);

found for $M^+ - (H_2O + HNCO)$ m/z 704.2711, $C_{35}H_{45}ClN_2O_{11}$ requires m/z 704.2711.

Treflorine (11). Band F, from preparative TLC of fraction B, gave substantially pure 11: 46 mg (1.7×10^{-4}% yield based on seed material); mp 205–208 °C dec (after recrystallization from CH_2Cl_2–hexane); IR ($CHCl_3$) 3600, 3440, 1760, 1715, 1675, 1640, 1610, 1590 cm^{-1}; UV_{max} (EtOH) 233 nm (ϵ 24000), 243 (sh, 18500), 253 (19850), 282 (5060), 288 (5060); $[\alpha]^{23}_D$ −138° (c 0.045, $CHCl_3$); mass spectrum (70 eV), m/z (relative intensity) 688 (M^+ – a, 2.7), 188 (5.7), 149 (4.2), 69 (14.7), 58 (32.1), 55 (13.4), 44 (100); found for $M^+ - (H_2O + HNCO)$ m/z 688.2751, $C_{35}H_{45}ClN_2O_{10}$ requires m/z 688.2762.

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Electronic Control of Stereoselectivity. 10. Aryl Substituent Effects on Electrophilic Stereoselection in 11-Isopropylidenedibenzonorbornadienes. Direct Competition of Dissimilarly Functionalized Benzo Groups[1]

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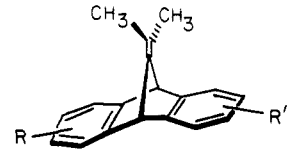
Three 11-isopropylidenedibenzonorbornadienes (**8**, **15a**, and **15b**) carrying dissimilarly substituted benzene rings have been prepared. To bypass a later need for product stereochemical assignment by X-ray analysis, we designed the synthetic schemes to be mediated by pure epoxide intermediates of spectroscopically definable syn configuration (**7**, **14a**, and **14b**). Perepoxidation, the prototypical weakly electrophilic reaction examined, gave rise in each instance to a pair of epoxide isomers whose identities could be readily established by spectral correlation with the precursor compounds. Ring opening of the six individual epoxide isomers with diethylaluminum 2,2,6,6-tetramethylpiperidide produced the corresponding isomerically pure allylic alcohols (**17**, **18**) whose ozonolysis furnished the structurally related α-hydroxy ketones (**19**, **20**). The chemical shifts of the methyl groups in these several types of compounds, although not widely divergent, proved to be sufficiently diagnostic of stereochemistry to permit reliable structural assignments to be made to the products of Friedel–Crafts acylation (**21**, **22**) and Prins hydroxymethylation (**23**, **24**). The syn/anti ratios observed for epoxidation are shown to correlate well with the relative abilities of the internally competing aromatic rings to enter into homoaromatic charge delocalization. When strong electrophiles are involved, the experimental data correlate most reasonably with the intervention of π complexes. Consequently, the latter reactions represent interesting examples of "guided" electrophile capture.

Previously, we reported our observations dealing with the addition of a variety of electrophiles to 9-isopropylidenebenzonorbornenes whose aryl substituents were varied in electronic character.[3] With weak electrophiles, a marked preference for contrathermodynamic anti attack was noted. In contrast, strong electrophiles were captured by the exocyclic double bond exclusively from the syn direction. These widely differing stereoselectivities were attributed to operation of long-range homoaromatic delocalization from the aromatic ring in the first instance (variably effective in proportion to available electron density) and to controlling steric accessibility in the latter situation. Second-order electronic influences were relegated to a significantly lesser role except for the possible involvement of coulombic interaction forces or charge-transfer complexation.

In a comparable study of benzobicyclo[2.2.2]octatriene derivatives where through-space coupling is nonoperational,[1] more subtle electronic effects surface, although a built-in steric bias persists. Understandably, our interpretative analysis of the nicely divergent directive influences showed by these two classes of compounds would be further substantiated if these stereoselectivity differences were to persist in the absence of steric imbalances. These requirements appeared to be met in the dibenzo systems **1** and **2** where the dissimilarly substituted aromatic rings are placed in direct competition. As matters turn out, 11-isopropylidenedibenzonorbornadienes (**1**) have been



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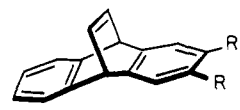

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little studied. Tanida and his co-workers have succeeded in preparing the parent hydrocarbon[4] and in demonstrating that its nitration with copper(II) nitrate and acetic anhydride in dichloromethane at room temperature gives rise to the 2-nitro derivative.[5] For our purposes, substrate

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