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ADDITIONAL NEW MAYTANSINOIDS FROM *TREWIA NUDIFLORA*: 10-EPITREWIASINE AND NORTREWIASINE[1]

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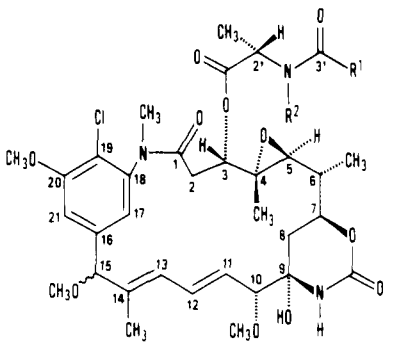
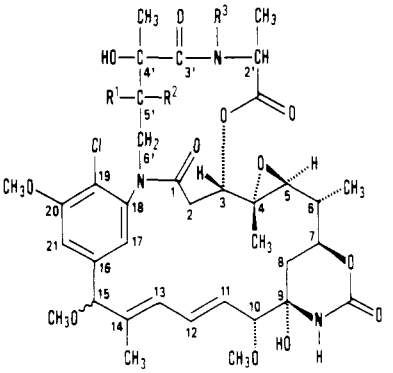
ABSTRACT.—Extracts of *Trewia nudiflora* (Euphorbiaceae) seed have previously yielded six new maytansinoids. These compounds, the most abundant of which is trewiasine (**1**), have potent tumor-inhibiting and insect-control properties in experimental systems. Further work has revealed two additional members of this series; 10-epitrewiasine (**7**) and nortrewiasine (**8**). Colubrinol (**9**), previously known only as a constituent of *Colubrina texensis* (Rhamnaceae), was also present. Isolation of the new maytansinoids was achieved by hplc, and structures were elucidated by ^{1}H-nmr, ^{13}C-nmr, and mass spectroscopy. In contrast to results with electron impact mass spectrometry, observation of molecular ions was achieved using negative ion chemical ionization mass spectrometry. Application of this technique to a series of previously characterized maytansinoids revealed that all give significant molecular anions.

In previous studies of *Trewia nudiflora* L. (Euphorbiaceae) seed, we reported structures of six new maytansinoids (1, 2). Included are trewiasine (**1**), dehydrotrewiasine (**2**), demethyltrewiasine (**3**), treflorine (**4**), trenudine (**5**), and *N*-methyltrenudone (**6**).





1 Trewiasine	R^1 = $CH(CH_3)_2$; R^2 = CH_3	
2 Dehydrotrewiasine	R^1 = $C(CH_3)=CH_2$; R^2 = CH_3	
3 Demethyltrewiasine	R^1 = $CH(CH_3)_2$; R^2 = H	
4 Treflorine	R^1 = R^2 = R^3 = H	
5 Trenudine	R^1 = OH; R^2 = R^3 = H	
6 *N*-Methyltrenudone	R^1 + R^2 = O; R^3 = CH_3	

These compounds are all cytotoxic to KB cells (*in vitro*), are active against PS leukemia (*in vivo*), and are toxic to or act as antifeedants against a variety of insect pests (3). Further investigation has revealed the presence of several minor constituents, including 10-epitrewiasine (**7**), nortrewiasine (**8**), and colubrinol (**9**). Wani *et al.* (4) previously characterized colubrinol as a constituent of *Colubrina texensis* (Rhamnaceae). Separation by hplc of the various *Trewia* maytansinoids is summarized in figure 1.

The electron impact mass spectrum (eims) of **7** exhibited an M^+-a ion[2] (5) having composition $C_{36}H_{49}ClN_2O_9$. Thus, it was apparent that **1** and **7** were isomeric, both

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[2]The eims of maytansinoids characteristically are devoid of molecular ions; however, distinctive ions are found at M^+ -61 [M^+ -(H_2O+HCNO), abbreviated as M^+ -a]. Elimination of the ester side-chain (b) at C-3 gives another distinguishing ion M^+ -(a+b) (5).

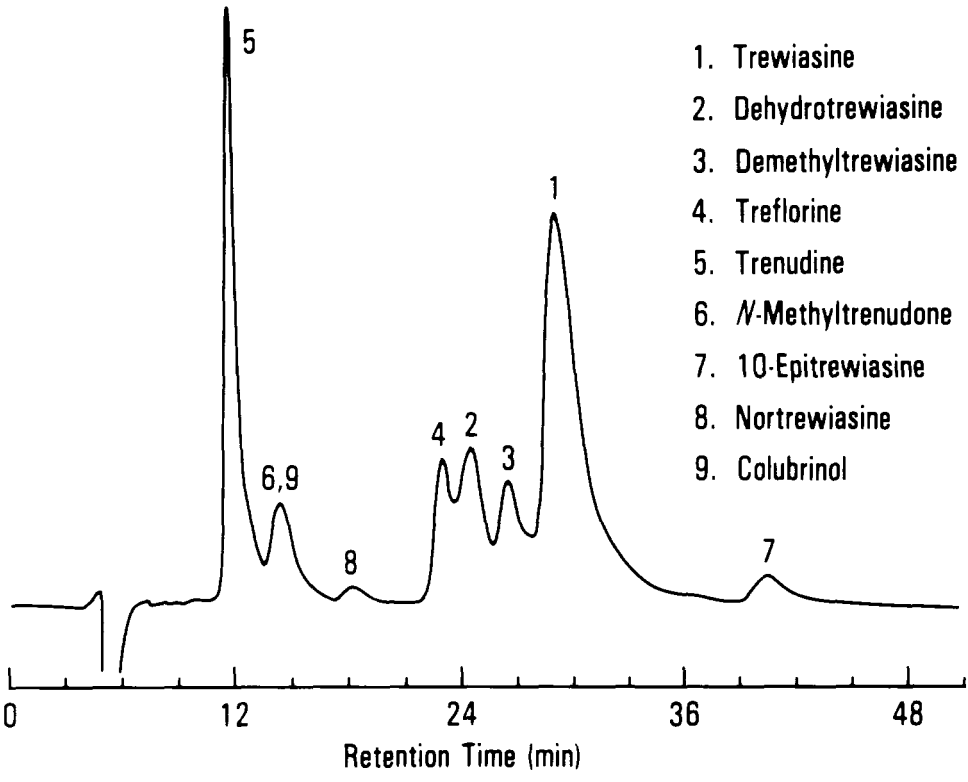
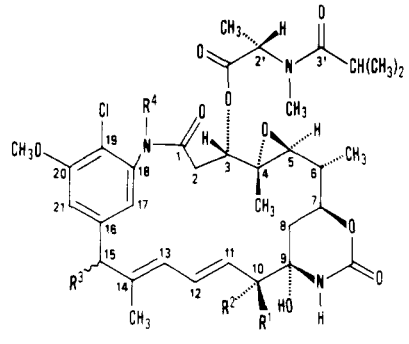


FIGURE 1. Hplc of *Treu'ia* maytansinoids on C_{18} μ-Bondapak using MeOH-H_2O (65:35). Mixture was prepared by recombination of *Treu'ia* maytansinoids in approximately the same proportions

having the molecular formula $C_{37}H_{52}ClN_3O_{11}$. Each compound gave another characteristic ion at *m/z* 515, M^+-(a+b), corresponding to elimination of the C-3 ester sidechain. Confirmation of the molecular weight of **7** was obtained by negative ion chemical ionization mass spectrometry (ni-cims), which gave a prominent molecular anion (M^-) at *m/z* 749. Comparison of the ^{1}H-nmr spectra of **1** and **7** (table 1) clearly demonstrates the close relationship of these two compounds. The only obvious difference in the two spectra involves protons assigned to positions 10 and 11. In **1**, the observed coupling constant between these two protons is 9.1 Hz, while in **7** the measured coupling constant is 3.5 Hz. Thus, **1** and **7** are clearly epimeric at C-10.



7	10-Epitrewiasine	$R^1 = OCH_3$; R^2 = H; $R^3 = OCH_3$; $R^4 = CH_3$
8	Nortrewiasine	R^1 = H; $R^2 = R^3 = OCH_3$; R^4 = H
9	Colubrinol	R^1 = H; $R^2 = OCH_3$; R^3 = OH; $R^4 = CH_3$

TABLE 1. ^{1}H-nmr of *Treuia nudiflora* Compounds[a]

Proton assignments	(470 MHz) Trewiasine (**1**)	(360 MHz) 10-Epitrewiasine (**7**)	(90 MHz) Nortrewiasine (**8**)	(360 MHz) Colubrinol (**9**)
2_A	2.18 dd	2.17 dd	2.25 dd	2.18 dd
	J=14.3, 3.0	J=14.4, 3.0	J=14.4, 3.0	J=14.3, 3.0
2_B	2.55 dd	2.58 dd	2.70 dd	2.56 dd
	J=14.3, 12.2	J=14.4, 12.2	J=14.4	J=14.3, 12.0
3	4.75 dd	4.83 dd	4.80 m	4.75 dd
	J=12.2, 3.0	J=12.2, 3.0		J=12.0, 3.0
4 CH_3	0.76 s	0.81 s	0.97 s	0.77 s
5	3.01 d	2.86 d	3.05 d	3.02 d
	J=9.6	J=9.6		J=9.6
6 CH_3	1.27 d	1.26 d	1.29 d	1.27 d
	J=6.2	J=6.3		J=6.1
7	4.28 m	4.23 m	4.33 m	4.27 m
10	3.51 d	3.72 d	3.55 d	3.51 d
	J=9.1	J=3.5		J=9.0
11	5.72 dd	5.90 dd	5.75 dd	5.71 dd
	J=15.3, 9.1	J=15.3, 3.5		J=15.3, 9.0
12	6.46 dd	6.49 dd	6.58 dd	6.45 dd
	J=15.3, 11.1	J=15.3, 11.1		J=15.3, 11.0
13	6.98 d	6.69 d	6.90 d	6.95 d
	J=11.1	J=11.1		J=11.0
14 CH_3	1.52 s	1.51 s	1.54 s	1.59 s
15	4.86 s	4.78 s	4.82 s	5.47 s
17	6.54 d	6.55 d	6.23 d	6.55 d
	J=1.5	J=1.5		J=1.5
21	7.22 d	7.23 d	7.22 d	7.34 d
	J=1.5	J=1.5		J=1.5
10 OCH_3[b]	3.35 s	3.36 s	3.38 s	3.34 s
15 OCH_3[b]	3.37 s	3.36 s	3.38 s	—
20 OCH_3	3.99 s	4.00 s	3.98 s	4.00 s
18 NCH_3	3.16 s	3.16 s	—	3.16 s
2′	5.37 m	4.80 m	5.34 m	5.36 m
2′ CH_3	1.28 d	1.34 d	1.29 d	1.26 d
	J=6.8	J=6.8		J=6.6
2′NCH_3	2.88 s	2.97 s	2.89 s	2.87 s
4′	2.76 m	2.75 m	2.76 m	2.76 m
4′CH_3	1.06 d	1.05 d	0.91 d	1.04 d
	J=6.6	J=6.6		J=6.5
4′CH_3	1.12 d	1.09 d	1.08 d	1.1 d
	J=6.8	J=6.8		J=6.8
NH	6.23 s	6.29 s	n. obs.	6.26 s

[a]Chemical shifts (δ) are expressed in ppm from tetramethylsilane, and coupling constants (J) are expressed in Hz. Extensive decoupling was used to verify assignments. Spectra were recorded in deuteriochloroform solution on a Fourier transform Brüker WH-90 spectrometer, on a Nicolet NT-360 spectrometer, or on a Nicolet NT-470 spectrometer as indicated. Assignments for **1** were reported earlier (1).

[b]These assignments may be reversed.

Comparison of the ^{13}C-nmr spectra of **1** and **7** (table 2) supports this conclusion in that the most significant differences in shifts occur in carbons assigned to C-10, or to carbons α and β to C-10 (C-8, C-9, C-11, and C-12) (6). An unexpected shift in the 2′ proton (upfield to δ 4.80) and a shift of the 2′ carbon (downfield to δ 53.81) may indicate that **1** and **7** are epimeric at C-2′. Conversely, the differences may be due to the various transannular shielding effects of the epimeric C-10 methoxyl groups. Epi-

TABLE 2. ^{13}C-nmr Assignments for Trewiasine (**1**) and 10-Epitrewiasine (**7**)[a]

Carbon assignments	Trewiasine (**1**)	10-Epitrewiasine (**7**)	Carbon assignments	Trewiasine (**1**)	10-Epitrewiasine (**7**)
2	32.43 t	32.62[b]	20	156.30 s	156.36
3	78.18 d	78.18	21	108.96 d	109.11
4	59.99 s	60.24	C=O	176.71 s	177.09
5	67.73 d	67.26	C=O	170.92 s	170.92
6	38.86 d	38.79	C=O	168.84 s	169.03
7	74.15 d	74.28	C=O	152.40 s	154.80
8	36.26 t	31.84[b]	OCH_3	56.3-7 3q	56.3-7, 58.4
9	80.72 s	82.73	CH_3	14.62 q	14.75
10	85.52 d	83.18	CH_3	13.13 q	13.25
11	129.92 d	128.81	CH_3	11.96 q	12.28
12	132.51 d	130.24	CH_3	10.01 q	10.07
13	127.97 d	126.21	18 NCH_3 . .	35.22 q	35.41
14	142.13 s	142.52	2′ NCH_3 . .	30.42 q	30.54
15	86.70 d	87.08	2′	52.38 d	53.81
16	141.35 s	141.41	4′	30.42 d	30.54
17	120.30 d	120.23	4′ CH_3 . .	19.43 q	19.23
18	139.01 s	136.86	4′ CH_3 . .	18.85 q	18.84
19	118.93 s	119.25			

[a]Chemical shifts (δ) are expressed in ppm from tetramethylsilane. Proton decoupled and off-resonance decoupled spectra were recorded in deuteriochloroform solution on a Fourier transform Brüker WH-90 spectrometer.

[b]Assignments may be reversed.

trewiasine (**7**) is unique in that all other known maytansinoids have the opposite configuration at C-10.

The ^{1}H-nmr spectrum of **8** lacked a signal at δ 3.16, characteristic of an *N*-methyl group at position 18, but was otherwise nearly identical to that of **1**. The eims of **8** gave an M^+-a ion at *m/z* 674 (CH_2 less than **1**) and an M^+-(a+b) signal at *m/z* 501. Ni-cims gave M^- at *m/z* 735. These observations are consistent only with structure **8**.

The ^{1}H-nmr spectrum of **9** lacked a signal for one of the three methoxyl groups common to all other *Trewia* maytansinoids (that at C-15), and the singlet attributed to H-15 was shifted downfield from δ 4.86 in **1**, to δ 5.47. Otherwise, the spectrum of **9** was nearly identical to that of **1**. The ^{1}H-nmr spectrum of **9** is quite similar to the published spectrum of colubrinol (7); in particular, the distinctive C-15 proton shift at δ 5.47 coincides nicely with the one recorded by Wall *et al.* The eims of **9** was similar to that of **8** [M^+-a, *m/z* 674 and M^+-(a+b), *m/z* 501] and ni-cims gave M^- at *m/z* 735, thus confirming that these two compounds are isomers. On the basis of these spectrometric data, we conclude that **9** is very probably identical with Wall's colubrinol. However, there is a considerable discrepancy between our values for the melting point and optical rotation of **9** and those reported by Wani *et al.* (4). Because of the very small sample remaining after biological testing, it would be impractical for us to attempt further recrystallizations of **9**.

Examination of the *Trewia* maytansinoids by ni-cims (isobutane) led to several interesting observations (table 3). Most importantly, we were able to obtain substantial molecular anions in every instance. Molecular ions are not normally observed in eims spectra of maytansinoids. Secondly, prominent peaks were consistently obtained for $(M-a)^-$ and, with the exception of compounds **4-6**, for $[M-(a+b)]^-$. Finally, in contrast to eims results, the fragment eliminated from C-3 could be directly observed as a major peak at $(b-H)^-$. In the spectra of **1, 7, 8,** and **9,** this negative ion was apparent at *m/z* 172 ($C_8H_{14}NO_3$). In **2**, this fragment was at *m/z* 170, while in **3** it was at *m/z* 158. In **4,**

TABLE 3. Negative Ion Chemical Ionization Mass Spectra of *Trewia* Maytansinoids

Compound	*m/z* (relative intensity)			
	M^-	$(M-a)^-$	$[M-(a+b)]^-$	$(b-H)^-$
1, trewiasine	749(41)	688(85)	515(31)	172(100)
2, dehydrotrewiasine	747(3)	686(100)	515(18)	170(17)
3, demethylrewiasine	735(1)	674(100)	515(14)	158(25)
4, treflorine	749(2)	688(100)	——	——
5, trenudine	765(1)	704(100)	——	——
6, *N*-methyltrenudone	777(1)	716(100)	——	——
7, 10-epitrewiasine	749(4)	688(100)	515(3)	172(10)
8, nortrewiasine	735(9)	674(59)	501(100)	172(23)
9, colubrinol	735(4)	674(100)	501(79)	172(47)

5, and **6**, the ester side-chain is incorporated into a second large ring; these compounds do not show the $(b-H)^-$ fragment nor do they show much fragmentation beyond the loss of $H_2O+HNCO$ (a) from the molecular anion. Some decomposition of maytansinoids under the conditions of evaporation from the ms probe was observed. The relative intensities of fragment peaks and the molecular anion (M^-) varied with heating profile. Best spectra were obtained with gentler heating profiles, and representative spectra were selected at the point of highest M^- signal. Certainly, the technique should prove useful in future studies of maytansinoids.

Cytotoxicity in the KB cell culture and *in vivo* PS activity of all the *Trewia* maytansinoids are summarized in table 4. It is apparent that epimerization at C-10 has little effect on biological activity in this series. Considerable information on the cytotoxicity and antileukemic activity of maytansine and its analogs has appeared in the literature (8, 9), and inactive compounds are obtained either by loss of the C-3 ester function or by etherification of the free hydroxyl group at C-9. Of taxonomic significance is the fact that maytansinoids are now known to occur in three plant families: Celastraceae, Rhamnaceae, and Euphorbiaceae. Certainly, there appears to be a close botanical relationship among these three families (10).

TABLE 4. Cytotoxic (KB) and Antileukemic (PS) Activity of *Trewia* Maytansinoids

Compound	KB cytotoxicity ED_{50}, μg/ml	Maximum PS activity T/C (dose, μg/kg)
1, trewiasine	2.0×10^{-4}	154(16)
2, dehydrotrewiasine	$<1.0 \times 10^{-2}$	172(8)
3, demethyltrewiasine	4.9×10^{-2}	159(8)
4, treflorine	2.7×10^{-4}	157(16)
5, trenudine	6.4×10^{-4}	146(8)
6, *N*-methyltrenudone	$<1.0 \times 10^{-2}$	153(4)
7, 10-epitrewiasine	$<1.0 \times 10^{-3}$	131(32)
8, nortrewiasine	$<1.0 \times 10^{-4}$	155(16)
9, colubrinol	$<1.0 \times 10^{-3}$	159(32)

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns block and are uncorrected. Analytical and preparative tlc were carried out on silica gel 60 F-254 plates (E. Merck), and plates were developed with CH_2Cl_2/MeOH (19:1). Hplc was carried out on a Waters Model ALC/PC-201 instrument equipped with a 7.8 X 400 mm C_{18} μ-Bondapak column, an RI detector, and operated at a flow rate of 2 ml/min. The 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. High-field (470 MHz or 360 MHz) ^{1}H-nmr spectra were obtained using Nicolet NT-470 or NT-360 instruments, and extensive decoupling was used to verify assignments. ^{1}H- (90 MHz) and ^{13}C- (22.63 MHz) nmr spectra were determined with a Brüker WH-90 instrument in $CDCl_3$ solution using tetramethylsilane as an internal standard. Mass spectra (eims) were measured using both Nuclide 12-90-DF and Kratos MS-30 instruments. Negative ion cims spectra were obtained using a Finnigan MAT 4535/TSQ instrument with isobutane as the reactant gas.

PLANT MATERIAL.—Seed of *Treuia nudiflora* (114 kg) was obtained in India (PR 53256 and PR 53445). The collection was arranged and authenticated by Dr. James Duke, USDA, Beltsville, MD, in accordance with the program developed by the National Cancer Institute.

EXTRACTION AND FRACTIONATION.—Extraction of *Treuia* seed, fractionation of the extract, and isolation of **1-6** by hplc were described earlier (1, 2). Fractions eluted after **1** were recombined and subjected to further hplc on a C-18 bonded column; this procedure yielded 40 mg of 10-epitrewiasine (**7**), with a retention time of 42 min. Similarly, fractions eluted between 17 and 21 min were rechromatographed, yielding 9 mg of nortrewiasine (**8**). Colubrinol (**9**) and *N*-methyltrenudone (**6**) eluted as a single hplc peak (figure 1), but were readily separated by preparative tlc; 50 mg of **9** was isolated in this manner (Rf 0.3 compared to Rf 0.4 for **6**).

10-EPITREWIASINE (**7**).—Final purification of **7** by preparative tlc and recrystallization from dichloromethane-hexane gave 40 mg (3.2×10^{-5}% yield); mp 159-162°; ir ($CHCl_3$) 3720, 3450, 1720, 1665, 1590 cm^{-1}; uv, λ max (EtOH) 233 nm (ε 21,700), 243 (sh, 17,200), 255 (18,350), 282 (5,060), 289 (5,060); $[\alpha]^{23}D$ −48° (*c* 0.103, $CHCl_3$); ^{1}H- and ^{13}C-nmr (see tables 1 and 2); eims, *m/z* (relative intensity): 688 (M^+ -a, 1), 653 (1), 515 (4), 500 (3), 484 (4), 480 (4), 448 (2), 191 (5), 156 (45), 128 (25), 58 (100), 44 (18), 43 (32). Found: M^+ -(a+b), *m/z* 515.2024; $C_{28}H_{34}ClNO_6$ requires 515.2074; negative ion cims (table 3).

NORTREWIASINE (**8**).—After preparative tlc and recrystallization from dichloromethane-hexane, 9 mg of **8** was obtained (7.2×10^{-6}% yield); mp 155-158°. $[\alpha]^{23}D$ −58° (*c* 0.04, $CHCl_3$);[3] ^{1}H-nmr (see table 2); eims, *m/z* (relative intensity): 674 (M^+ -a, 0.3), 501 (0.3), 58 (100), 44 (50), 43 (63), 41 (38). Found: M^+ -(a+b), *m/z* 501.1930; $C_{27}H_{32}ClNO_6$ requires 501.1918; negative ion cims (table 3).

COLUBRINOL (**9**).—Final purification by preparative tlc followed by crystallization from dichloromethane-hexane gave **9**, 50 mg (4.0×10^{-5}% yield); mp 174-178° (d); Lit. 194-196° (4); ir ($CHCl_3$) 3620, 3450, 1750, 1715, 1665, 1640, 1590 cm^{-1}, uv, λ max (EtOH) 233 nm (ε 17,100), 243 (sh, 14,000), 253 (14,700), 280 (4,040), 289 (3,860); $[\alpha]^{23}D$ −70°, (*c* 0.555, $CHCl_3$); [Lit. $[\alpha]^{22}D$ −94° (4)]; ^{1}H-nmr (see table 2); eims, *m/z* (relative intensity): 674 (M^+ -a, 0.1), 501 (1.0), 58 (86), 55 (50), 44 (100), 43 (61), 41 (55). Found: M^+ -(H_2O+HNCO+$C_8H_{15}NO_3$), *m/z* 501.1900; $C_{27}H_{32}ClNO_6$ requires 501.1918; negative ion cims (table 3).

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[3]It is probable that our melting point and $[\alpha]D$ are somewhat inaccurate because we observed the rapid decomposition of **8** as it stood in solvent at room temperature. Similar behavior has been reported for normaytansine (**11**).

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