NORMAYTANCYPRINE, A MINOR ANTILEUKEMIC ANSA MACROLIDE FROM *PUTTERLICKIA VERRUCOSA*

ALBERT T. SNEDEN[1]

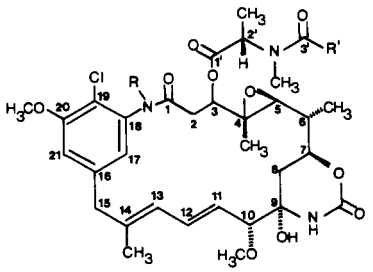
Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284

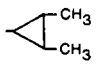
WILLIAM C. SUMNER, JR. and S. MORRIS KUPCHAN[2]

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901

ABSTRACT.—Normaytancyprine (**2**), a new minor antileukemic maytansinoid, was isolated from *Putterlickia verrucosa* Szyszyl. The structure was elucidated by a chemical interconversion to normaysine and by ^1H and ^{13}C nmr and mass spectrometry. Normaytancyprine was found to contain an unusual dimethylcyclopropylcarboxyl moiety in the C-3 amino acid ester.

Maytansine **1**, a potent antileukemic ansa macrolide originally isolated from *Maytenus serrata* (Celastraceae), has undergone several clinical trials under the auspices of the National Cancer Institute (1,2). Because of the low yield of **1** from *M. serrata* (*ca.* 0.0001%), several other plants in the Celastraceae family have been assayed for maytansine **1** content (3). During the course of the isolation of **1** from *Putterlickia verrucosa* Szyszyl. (Celastraceae), a new minor antileukemic maytansinoid, normaytancyprine **2**, has now been isolated.[3,4]



	R	R'
1	$-CH_3$	$-CH_3$
2	-H	
3	-H	$-CH_3$

An ethanolic extract of 60 kg of the dried stem wood and stem bark of *P. verrucosa* was subjected to the isolation procedure developed for *Maytenus buchananii* (3). Extensive column chromatography and preparative thin layer chromatography (ptlc) of the appropriate maytansinoid-containing fractions led to the isolation of several maytansinoids[3] and 6 mg (0.00001%) of **2** as pale tan microcrystals, mp 143–145°.

The ultraviolet spectrum of **2** had absorption maxima at 232, 252, 280, 289 nm and the infrared spectrum had bands at 1750, 1710, and 1635 cm^{-1}, suggesting that **2** was a maytanside ester. The mass spectrum was typical of a maytanside ester (2,4) with the highest observable ion at *m/e* 670 corresponding to M^+-H_2O-HNCO. The pattern of ions at *m/e* 471, 456, and 436 was indicative of the

[1]Author to whom inquiries should be directed.

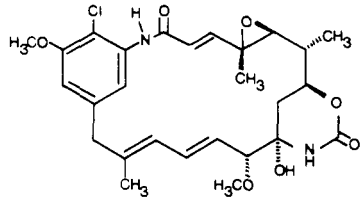
[2]Deceased, October 19, 1976.

[3]The isolation of maytansine **1** and other maytansinoids from *P. verrucosa* was previously reported in Reference 3.

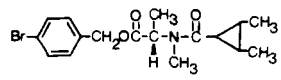
[4]Normaytancyprine **2** was previously isolated in small amounts from large scale extracts of *M. buchananii*, but the structure was never elucidated.

macrocyclic ring of an 18-*N*-demethylmaytansinoid [cf. normaytansine **3** (4)], and the loss of 199 mass units from the *m/e* 670 ion suggested that the C(3) side chain ester was derived from an amino acid with the molecular formula $C_{10}H_{17}NO_3$.

The ^{1}H nmr spectrum of **2** confirmed that **2** was an 18-*N*-demethylmaytansinoid since the singlet due to an 18-*N*-methyl group was missing from the spectrum. All other characteristic signals for the macrocyclic ring were evident in the spectrum. Confirmation of the structure of the macrocyclic ring system was obtained by hydrolysis of **2** with 30% aqueous potassium hydroxide to yield normaysine **4**, identical with an authentic sample by tlc and by comparison of ir and ^{1}H nmr spectra.



4



5

A doublet at δ1.28, a quartet at δ5.29, and a singlet at δ2.90 in the ^{1}H nmr spectrum of **2** indicated that the C(3) side chain was a derivative of *N*-methyl-L-alanine as found in other maytansinoids. However, the remaining signals attributed to the side chain appeared between δ0.6 and δ2.2 and assignment of individual signals was not possible. To aid in the structure determination of the side chain, the *p*-bromobenzyl ester **5** of the side chain acid (obtained from the alkaline hydrolysis of **2**) was prepared. The ester was obtained as an oil and the ^{1}H nmr spectrum did not define the structure of the *N*-acyl group. However, the mass spectral fragmentation pattern of **5** (figure 1) was very helpful.

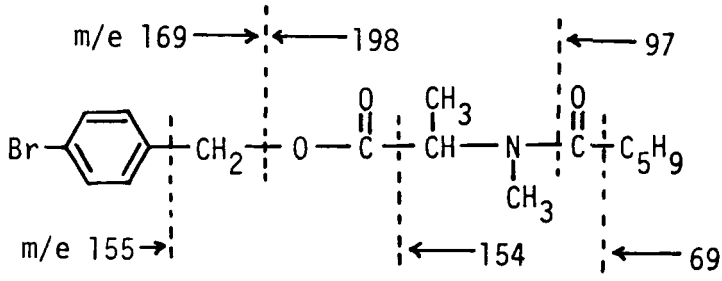


FIGURE 1. Mass spectral fragmentation pattern of the p-bromobenzyl ester **5** of the C3-side chain acid of normaytancyprine **2**.

The parent ion at *m/e* 367 suggested the molecular formula $C_{17}H_{22}NO_3Br$, which agreed with the molecular formula $C_{10}H_{17}NO_3$ for the acid suggested previously. It was known that this acid was an *N*-acyl derivative of *N*-methyl-L-alanine from the ir and ^{1}H nmr spectra of **2** and **5**, and the ions at *m/e* 198, 182, and 154 in the mass spectrum of **5** supported this conclusion. The ions at *m/e* 97 and 69 indicated that the acyl moiety had the formula C_6H_9O, which implied the presence of one double bond or one ring in this fragment. Since no vinyl protons or vinyl methyl groups were evident in the ^{1}H nmr spectrum, the only possibility was a carbocyclic ring. Careful reexamination of the ^{1}H nmr spectra of **2** and **5** revealed

two methyl groups between δ0.6 and δ2.0. Thus, a dimethyl cyclopropanecarboxylic acid derivative was considered to be the most likely possibility.

Elucidation of the structure of **2** was finally accomplished by comparison of its ^{13}C nmr spectra with those of maytansine **1** and normaysine **4** (table 1) (5,6). The major differences between the spectra of **1** and **2** were the absence of two quartets due to the (18)*N*–CH_3 and the C(4')H_3 in the spectra of **2**. These signals were replaced in the spectra of **2** by five new signals, three doublets at 20.3, 23.3, and 27.5 ppm and two quartets at 12.2 and 17.4 ppm. These resonances in conjunction with the mass spectral data could be explained only by a 2,3-dimethylcyclopropanecarboxylic acid derivative as the *N*-acyl moiety of the side chain.

TABLE 1. ^{13}C nmr chemical shifts (ppm) of **1**, **2**, and **4**.

Carbon Assignment	1	2	4
2	32.5 t	32.6 t	118.8 d
3	78.2 d	78.2 d	150.0 d
4	60.1 s	60.2 s	59.8 s
5	67.2 d	66.9 d	66.9 d
6	39.1 d	39.0 d	39.1 d
7	74.2 d	74.4 d	74.8 d
8	36.5 t	36.2 t	35.8 t
9	81.0 d	81.1 s	81.1 s
10	88.9 d	88.5 d	88.5 d
11	127.8 d	128.4 d	127.9 d
12	133.3 d	133.0 d	132.4 d
13	125.4 d	127.0 d	125.8 d
14	139.1 s[a]	135.8 s[a]	139.2 s[a]
15	46.7 t	46.6 t	46.6 t
16	142.4 s[a]	140.7 s[a]	139.6 s[a]
17	122.5 d	120.3 d	120.8 d
18	141.2 s[a]	138.3 s[a]	135.7 s[a]
19	119.1 s	116.1 s	115.6 s
20	156.1 s	155.8 s[b]	156.0 s[b]
21	113.4 d	112.2 d	111.4 d
C=O	152.2 s[b]	152.4 s[b]	152.4 s[b]
C=O	168.8 s	170.4 s	164.6 s
C=O	170.2 s	171.3 s	—
C=O	170.8 s	171.5 s	—
4–CH_3	12.2 q	12.9 q	14.3 q
6–CH_3	14.5 q	14.6 q	14.9 q
14–CH_3	15.5 q	15.9 q	16.0 q
10–OCH_3			
20–OCH_3	56.7 2q	56.7 2q	56.6 2q
18–NCH_3	35.4 q	—	—
2'	52.2 d	52.9 d	—
2'–CH_3	13.4 q	13.7 q	—
2'–NCH_3	31.7 q	30.9 q	—
4'	21.7 q	20.3 d[c]	—
5'	—	23.2 d[c]	—
6'	—	27.5 d[c]	—
5'–CH_3	—	17.4 q[d]	—
6'–CH_3	—	12.2 q[d]	—

[a,b,c,d]May be reversed in any column.

All spectra were recorded at 22.5 MHz in $CDCl_3$ with $(CH_3)_4Si$ as an internal standard.

Thus, based upon the accumulated chemical and spectral evidence, normaytancyprine was assigned structure **2**. No assignment of stereochemistry in the *N*-acyl moiety was possible from the available evidence. Normaytancyprine **2** was found to exhibit significant *in vivo* activity against the P388 lymphocytic leukemia in mice at doses comparable to maytansine **1** (T/C=145–300% at

0.4–12.5 μg/kg) and cytotoxicity against the KB cell culture (ED_{50} 10^{-5}–10^{-6} μg/ml) (7).[5]

EXPERIMENTAL[6]

PLANT MATERIAL.—Dried stem wood and stem bark of *Putterlickia verrucosa* Szyszyl. (B628207, PR-41187) collected in South Africa in 1972 was supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, where voucher specimens are preserved.

ISOLATION OF NORMAYTANCYPRINE (**2**).—The dried, ground plant material (60 kg) was extracted in five 12 kg batches with 95% ethanol (Soxhlet) and subjected to a slightly modified "Improved Fractionation Procedure for *Maytenus buchananii*" as described in Reference 3. The maytansinoid-containing fraction from column chromatography over alumina was subjected to column chromatography over silica gel 60 (EM Labs) and eluted with dichloromethane followed by increasing amounts of methanol in dichloromethane. Fractions from this column were combined according to tlc. Two similar fractions which eluted with 2% methanol in dichloromethane were combined and subjected to successive ptlc in the following systems; tlc plates (developing solvent): ChromAR (Mallinckrodt) (20% benzene in ethyl acetate, 2X); Silica gel 60 (20% benzene in ethyl acetate, 3X); silica gel 60 (20% benzene in ethyl acetate, 2X); alumina (EM Labs) (6% methanol in benzene, 2X); silica gel 60 (4% methanol in chloroform, 3X). The material obtained from the final ptlc separation was isolated as a clear glass and crystallized from chloroform-hexanes to yield 6 mg **2**, 0.00001%; mp 143–145°; uv max (EtOH) 232 nm (ε 48700), 252 (28250), 280 (7450), 289 (7420); ir ($CHCl_3$) 3700, 3420, 3390, 2940, 1750, 1710, 1635, 1605, 1455, 1395, 1330, 1310, 1168, 1100, 1085 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.64 (3H, d, J=5 Hz, C5' or C6'-CH_3), 1.00 (3H, s, C4-CH_3), 1.28 (6H, 2d, J=7 Hz, C6-CH_3, C2'-CH_3), 1.66 (3H, brs, C14-CH_3), 2.27 (1H, dd, $J_{2,3}$=2.7 Hz, $J_{2,2}$=14 Hz, C2-H), 2.90 (3H, s, C2'-NCH_3), 3.08 (1H, d, $J_{5,6}$=9 Hz, C5-H), 3.10 (1H, d, $J_{15,15}$=14 Hz, C15-H), 3.36 (3H, s, C10-OCH_3), 3.52 (1H, d, $J_{10,11}$=8.6 Hz, C10-H), 3.69 (1H, d, $J_{15,15}$=14 Hz, C15-H), 3.95 (3H, s, C20-OCH_3), 4.26 (1H, m, C7-H), 4.86 (1H, dd, $J_{2,3}$=2.7, 11 Hz, C3-H), 5.29 (1H, q, J=7 Hz, C2'-H), 5.75 (1H, dd, $J_{10,11}$=8.6 Hz, $J_{11,12}$=14 Hz, C11-H), 6.38 (1H, brs, C9-NH), 6.52 (1H, dd, $J_{11,12}$= 14 Hz, $J_{12,13}$=11 Hz, C12-H), 6.70 (1H, d, $J_{12,13}$=11 Hz, C13-H), 6.76, 6.90 (1H ea, 2 d, J=1.5 Hz, C17-H, C21-H), 7.47 (1H, brs, C18-NH), 0.8–2.1 (10H, m, C2-H, C6-H, C8-H_2, C4'-H, C5'-H, C6'-H, C5' or C6'-CH_3); mass spectrum *m/e* 670, 471, 456, 436, 182, 154, 97, 69; FAB high resolution mass spectrum *m/e* 732.3256 (calc'd for $C_{37}H_{50}N_3O_{10}Cl+H^+$ 732.3262).

HYDROLYSIS OF **2**.—A solution of 15.7 mg of **2** in 30% aqueous KOH was stirred at 25° for 10 min. The solution was diluted with 2 ml of water and extracted with dichloromethane (6 x 5 ml). The combined dichloromethane layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give 7.2 mg of residue. Ptlc of this residue on silica gel 60 eluted with 5% methanol in chloroform (2X) gave 2.6 mg of normaysine **4**, identical with authentic **4** by ir, ^1H nmr, and tlc.

The aqueous layer was cooled in ice and acidified with 10 drops of conc. HCl. The solution was extracted with dichloromethane (6 x 5 ml), and the combined dichloromethane layers were dried over anhydrous Na_2SO_4. The dichloromethane was removed *in vacuo* to give 5.9 mg of residue.

PREPARATION OF *p*-BROMOBENZYL ESTER **5**.—The residue from the acid layer above was dissolved in 1 ml of dimethylformamide, and 23.6 mg Ag_2O and 16.8 mg *p*-bromobenzylbromide were added. The mixture was stirred at room temperature for one hour, filtered through Celite, and concentrated *in vacuo*. The residue, when subjected to ptlc on ChromAR developed with 5% methanol in ethyl acetate followed by ptlc of the major band on alumina developed with 5% methanol in ethyl acetate (2X), yielded 3.9 mg **5** as a yellow oil: ir (CCl_4) 2980, 2950, 2890, 1760, 1660, 1500, 1465, 1200, 1130, 1100 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.02 (3H, s, N-CH_3), 5.10 (2H, s, $ArCH_2O$-) 5.20 (1H, q, J=7 Hz, C2-H), 7.21, 7.49 (2H, ea 2d, J=8.3 Hz, Ar-H_4) 0.8–1.5 (11H, m, C2-CH_3, C5-CH_3, C4-H, C6-CH_3, C6-H, C5-H); mass spectrum *m/e* 369 (M^++2), 367 (M^+), 198 ($M^+-C_7H_6Br$), 169 (C_7H_6Br), 155 (C_6H_5Br), 154 ($C_9H_{16}NO$), 97 (C_6H_9O), 69 (C_5H_9).

ACKNOWLEDGMENTS

This work was supported by the Grants-In-Aid Program for Faculty of Virginia Commonwealth University (ATS) and a National Cancer Institute Contract (NO1-CM-67002) (SMK). We thank Dr. John M. Cassady, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, and, particularly, Dr. John Occolowicz, Eli Lilly Co. for obtaining the high resolution mass spectrum of **2**.

Received 12 February 1982

[5]The P388 lymphocytic leukemia *in vivo* assays were performed at Raltech Associates, Madison, Wisconsin, and the KB cytotoxicity assays were performed at Arthur D. Little, Inc., Cambridge, Massachusetts.

[6]All mps are uncorrected. ^1H and ^{13}H C nmr spectra were recorded on a JEOL FX90Q spectrometer in deuterochloroform with $(CH_3)_4Si$ as an internal standard. The ir spectra were measured on a Perkin-Elmer model 283 instrument and the uv spectra were measured on a Beckman Acta MVII recording spectrophotometer. Low resolution mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Center.

LITERATURE CITED

1. S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, and R. F. Bryan, *J. Amer. Chem. Soc.*, **94**, 1354 (1972).
2. J. Douros and M. Suffness, *Cancer Chemother. Pharmacol.*, **1**, 91 (1978).
3. S. M. Kupchan, Y. Komoda, A. R. Branfman, A. T. Sneden, W. A. Court, G. J. Thomas, H. P. J. Hintz, R. M. Smith, A. Karim, G. A. Howie, A. K. Verma, Y. Nagao, R. G. Dailey, Jr., V. A. Zimmerly, and W. C. Sumner, Jr., *J. Org. Chem.*, **42**, 2349 (1977).
4. A. T. Sneden and G. L. Beemsterboer, *J. Nat. Prod.*, **43**, 637 (1980).
5. W. A. Wallace and A. T. Sneden, *Org. Magn. Reson.*, **19**, 31 (1982).
6. R. G. Powell, D. Weisleder, and C. R. Smith, Jr., *J. Org. Chem.*, **46**, 4398 (1981).
7. R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).