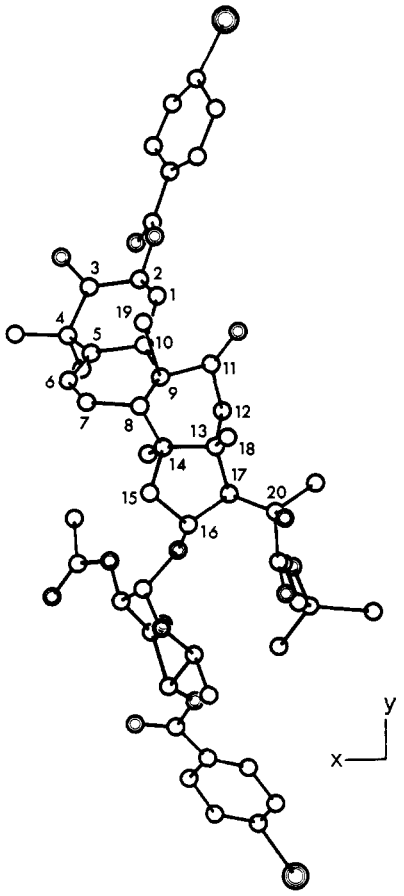

Figure 1. Molecular structure of the di-*p*-iodobenzoate of datiscoside as found in the crystal. Oxygen atoms are denoted by the double circles, the iodine atoms by the large circles. The axial system shown is right-handed and the molecule is shown with its correct absolute configuration.

the mirror image of **1** yielded $R = 0.124$ indicating a significant discrimination between the two enantiomers.[11] The correctness of the assignment of the absolute configuration was further confirmed by the measurement of a substantial number of Friedel pairs of reflections where, in all cases, the observed difference in intensity was in good agreement with theoretical expectation.[12]

The glycoside moiety at C-16 of datiscoside has thus been characterized as a novel 2′-*O*-acetyl-6′-deoxy-α-L-*gluco*-hexos-3′-ulopyranoside.[13] The β-equatorial configuration for the C-2 substituent of dihydrocucurbitacin D acetate had been correctly assigned by Lavie, *et al.*,[4] on the basis of the nmr signal for the C-2 proton [τ 4.4 (dd, J = 13.5, 5.1 Hz)]. A corresponding signal appeared in the nmr spectrum of datiscoside diacetate. The CD-based arguments which led to proposal of the opposite configuration require further study.[5,13a]

(11) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).

(12) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature (London)*, **168**, 271 (1951).

(13) See W. Pigman and D. Horton, Ed., "The Carbohydrates," 2nd ed, Vol. IIB, Academic Press, New York, N. Y., 1970, pp 809–833, for nomenclature.

(13a) NOTE ADDED IN PROOF. The present work confirms the 2β,3β-diol configuration in cucurbitacins O, P, and Q [S. M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970)], in view of the demonstrated cis configuration of their 2,3-diol system and of their interrelation with cucurbitacin B [S. M. Kupchan, R. M. Smith, Y. Aynehchi, and M. Maruyama, *J. Org. Chem.*, **35**, 2891 (1970)].

The observed antileukemic and tumor-inhibitory activity of datiscoside (**1**) confirms and extends an earlier report of antitumor activity of a cucurbitacin glycoside.[14] Investigations are in progress to determine the significance of the glycoside and of other structural features to the tumor-inhibitory activity of datiscoside.

(14) D. Lavie, D. Willner, M. Belkin, and W. G. Hardy, *Acta Unio. Int. Contra Cancrum*, **15**, 177 (1959); H. El Khadem and M. M. A. Abdel Rahman, *J. Chem. Soc.*, 4991 (1963).

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Maytansine, a Novel Antileukemic Ansa Macrolide from *Maytenus ovatus*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors from plant sources, we found that an alcoholic extract of *Maytenus ovatus* Loes.[3] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) and against five standard animal tumor systems.[4] We report herein the isolation and structural elucidation of maytansine (**1**), a novel antileukemic ansa macrolide tumor inhibitor[5] from *Maytenus ovatus*. Maytansine is the first ansa macrolide shown to contain carbinolamine, epoxide, or aryl halide functions and appears to be the first member of the series reported to show significant *in vivo* tumor inhibitory activity.

Fractionation of the alcohol extract, guided by assay against KB and P-388, revealed that the inhibitory activity was concentrated, successively, in the ethyl acetate layer of an ethyl acetate–water partition and in the methanol layer of a 10% aqueous methanol–petroleum ether partition. Column chromatography on SilicAR CC7 followed by treatment with acetic anhydride–pyridine[6] and extensive column chromatography and preparative tlc on alumina, silica gel, and SilicAR afforded a highly enriched concentrate (A, 1 mg/kg of plant). Attempts to prepare different derivatives in methanol solution yielded a common product, apparently a methyl derivative, **2**, whereas similar experiments in ethanol solution yielded a common ethyl derivative, **3**. Accordingly, concentrate A was treated at room temperature with 3-bromopropanol-

(1) Tumor Inhibitors. LXXIII. Part LXXII: S. M. Kupchan, C. W. Sigel, L. J. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, **94**, 1353 (1972).

(2) Supported by grants from the National Cancer Institute (NCI, CA-11718) and American Cancer Society (T-275 and T-541), and a contract with Chemotherapy, National Cancer Institute (NIH 71-2099).

(3) Fruits were collected in Ethiopia in Jan 1962. Roots and the wood of stems from Ethiopia and Kenya also yielded active extracts. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.

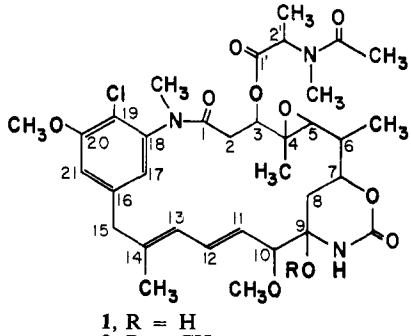
(4) Activity was noted against sarcoma 180, Lewis lung carcinoma, and L-1210 and P-388 leukemias in the mouse and Walker 256 intramuscular carcinosarcoma in the rat. Cytotoxicity and *in vivo* activity were assayed as in *Cancer Chemother. Rep.*, **25**, 1 (1962).

(5) Maytansine showed significant antileukemic activity against P-388 lymphocytic leukemia over a 50–100-fold dosage range at the microgram per kilogram level, and cytotoxicity (ED_{50}) against KB cell culture at 10^{-4}–10^{-5} μg/ml.

(6) Pilot experiments revealed that acetylation facilitated the subsequent separation without affecting maytansine.

1 and TsOH in CH_2Cl_2, to yield the carbinolamine ether (3-bromopropyl)maytansine (**4**): $C_{37}H_{51}BrClN_3O_{10}$; mp 176–178°; ir (KBr) 5.76, 6.01, 6.34, 8.42, 9.29 μ. Treatment of **4** with 2 *N* HCl in aqueous methanol afforded a crystalline hydrolysis product,[7] which was used to seed a solution of concentrate A in ether–CH_2Cl_2 to yield maytansine (**1**) (0.2 mg/kg of plant, 0.00002%): $C_{34}H_{46}ClN_3O_{10}$; mp 171–172°; $[\alpha]^{26}D$ −145° (*c* 0.055, $CHCl_3$); uv max (EtOH) 233 (ϵ 29,800), 243 (sh, ϵ 27,100), 254 (ϵ 27,200), 282 (ϵ 5690), 290 nm (ϵ 5520); ir (KBr) 5.75, 5.80, 6.02, 6.34, 8.42, 9.26 μ; mass spectrum *m/e* 630.2680, $C_{33}H_{43}ClN_2O_8$ [M −61 (H_2O + HNCO[8])] = 630.2708; nmr ($CDCl_3$) τ 9.13 (3 H, s, C-4–CH_3), 8.66 (3 H, d, J = 6 Hz, C-6–CH_3), 8.63 (3 H, d, J = 7 Hz, C-2′–CH_3), 8.31 (3 H, broad s, C-14–CH_3), 7.85 (3 H, s, C-2′N–$COCH_3$), 7.79 (1 H, d of d, $J_{2,2}$ = 15, $J_{2,3}$ = 3 Hz, C-2–H), 7.35 (1 H, d of d, $J_{2,2}$ = 15, $J_{2,3}$ = 12 Hz, C-2–H), 7.11 (3 H, s, C-2′N–CH_3), 6.96 (1 H, d, $J_{5,6}$ = 9 Hz, C-5–H), 6.87 (1 H, d, $J_{15,15}$ = 13 Hz, C-15–H), 6.78 (3 H, s, C-1N–CH_3), 6.62 (3 H, s, C-10–OCH_3), 6.50 (1 H, d, $J_{10,11}$ = 9 Hz, C-10–H), 6.47 (1 H, s, OH), 6.33 (1 H, d, $J_{15,15}$ = 13 Hz, C-15–H), 6.01 (3 H, s, C-20–OCH_3), 5.72 (1 H, m, C-7–H), 5.21 (1 H, d of d, $J_{2,3}$ = 12, 3 Hz, C-3–H), 4.65 (1 H, q, J = 7 Hz, C-2′–H), 4.34 (1 H, d of d, $J_{10,11}$ = 9, $J_{11,12}$ = 15 Hz, C-11–H), 3.76 (1 H, broad s, C-9N–H), 3.58 (1 H, d of d, $J_{11,12}$ = 15, $J_{12,13}$ = 11 Hz, C-12–H), 3.30 (1 H, broad d, $J_{12,13}$ = 11 Hz, C-13–H), 3.25, 3.16 (2 H, d, $J_{17,21}$ = 1.5 Hz, C-17–H, C-21–H), 9.20–7.50 (3 H, C-6–H, C-8–H_2).

The reversible interrelation of maytansine (**1**) and the 3-bromopropyl derivative **4** made the latter compound an attractive target for X-ray crystallographic analysis. Crystals of **4** belong to the orthorhombic system with space group $P2_12_12_1$ and have a = 24.239 (4), b = 16.044 (4), c = 10.415 (2) Å. The unit cell contains four formula units of $C_{37}H_{51}BrClN_3O_{10}$, giving a calculated density of 1.34 g/cm³, in reasonable agreement with the observed value of 1.30 g/cm³. By diffractometry, using monochromatic Cu Kα radiation, scintillation counting, and pulse-height analysis, 952 independent intensities significantly above background were recorded.



1, R = H
2, R = CH_3
3, R = CH_3CH_2
4, R = $BrCH_2CH_2CH_2$

The structure was solved by the heavy-atom method. Refinement of the structural parameters was by block-diagonal least-squares methods using anisotropic thermal parameters only for the bromine atom and, following its identification in the molecule, the chlorine atom, to yield R = 0.101.

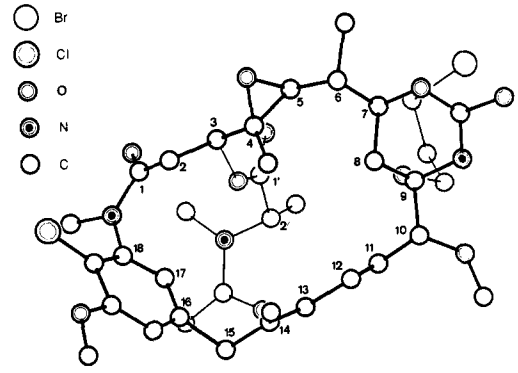


Figure 1. View of the molecular structure of (3-bromopropyl)-maytansine (**4**) as found in the crystal. The projection is onto the least-squares mean plane through the 19 atoms of the macrocyclic ring and the molecule is drawn in its correct absolute configuration with respect to a right-handed axial system. The numbering scheme corresponds to that used in the structural formula shown in the text.

The assignment of absolute configuration was done by taking into account the anomalous scattering of the bromine and chlorine atoms. When $\Delta f''$ was included in the structure factor calculation the two alternate enantiomers yielded R = 0.103 and 0.101, a significant difference at the 99% level.[9] Confirmation of the assignment was obtained by comparison of the observed intensity for 23 Friedel pairs of reflections. In each case the difference in intensity was in good agreement with that calculated for the chosen enantiomer.[10]

The structure found for the molecule in the crystal is shown in Figure 1 and leads unequivocally to the structural interpretation in **4**. The absolute configurations are 3*S*, 4*S*, 5*S*, 6*R*, 7*S*, 9*S*, 10*R*, and 2′*S*.

The disposition of substituents about the various bond axes shows almost perfect minimization of the intramolecular repulsions so that no strong intermolecular forces seem to be involved in dictating the observed conformation of the molecule. The calculated esd in an atomic position is around 0.025 Å, corresponding to an esd in bond distance of about 0.04 Å and in bond angle of about 3°. The rms deviation for equivalent C–C distances is closer to 0.06 Å suggesting that the errors calculated from the least-squares matrices are underestimated. Thermal parameters in the macrocycle are physically reasonable, being between 2.6 and 8.0 Å², but the substituent groups show stronger thermal vibrations with the bromine atom having an equivalent isotropic thermal parameter of 11 Å².

Maytansine's ansa macrolide structure shows noteworthy similarities to those of the rifamycins,[11] streptovaricins,[12] tolypomycins,[13] and geldanamycin.[8,14] The ansamycin antibiotics and their derivatives have

(7) The identity was confirmed by direct comparison with maytansine.
(8) K. Sasaki, K. L. Rinehart, Jr., G. Slomp, M. F. Grostic, and E. C. Olson, *J. Amer. Chem. Soc.*, **92**, 7591 (1970).
(9) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).
(10) J. M. Bijvoet, A. F. Peerdeman, and A. T. van Bommel, *Nature (London)*, **168**, 271 (1951).
(11) W. Oppolzer, V. Prelog, and P. Sensi, *Experientia*, **20**, 336 (1964).
(12) K. L. Rinehart, Jr., M. L. Maheshwari, F. J. Antosz, H. H. Mathur, K. Sasaki, and R. J. Schacht, *J. Amer. Chem. Soc.*, **93**, 6273 (1971); A. H.-J. Wang, I. C. Paul, K. L. Rinehart, Jr., and F. J. Antosz, *ibid.*, **93**, 6275 (1971).
(13) T. Kishi, S. Harada, M. Asai, M. Muroi, and K. Mizuno, *Tetrahedron Lett.*, 97 (1969).
(14) Experiments are underway to evaluate the possibility that microorganisms may play a role in the biosynthesis of maytansine.

aroused considerable interest as antiviral and antimicrobial agents, and as inhibitors of RNA tumor virus reverse transcriptases.

Investigations are in progress to determine the potential significance of the carbinolamine, epoxide, aryl chloride, and other structural features in relation to the biological activity of maytansine.

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Carbon to Metal Chlorine Exchange. III. Mercuric Chloride Promoted Reactions of *exo*-Norbornyl Chloride[1]

Sir:

In the $HgCl_2$-promoted reactions of *p*-chlorobenzhydryl chloride (RCl) in anhydrous acetone,[2,3] the rate of racemization (k_{rac}) of optically active RCl proceeds $^3/_2$ times faster than the rate of chlorine exchange (k_e) with $HgCl_2$ containing radiolabeled chlorine. The explanation offered was that RCl was regenerated from *racemic* $R^+HgCl_3^-$ ion pairs, I, in which all three chlorine atoms on mercury are *equivalent* but so constituted that two chlorine atoms are from the originally labeled $HgCl_2$ and one is from the RCl. When the solvent is changed to 80% acetone,[3] *ca.* 55% of the ion pair intermediates dissociate and collapse with water to produce the ROS product. However, the ion pair intermediates which regenerate RCl have the same constitution as in anhydrous acetone.

$$d\text{-RCl} \longrightarrow dl\text{-RCl} \qquad k_{rac}$$
$$d\text{-RCl} + \text{SOH} \longrightarrow dl\text{-ROS} + \text{HCl} \qquad k_t \quad \Big\} k_\alpha$$
$$\text{RCl} + HgCl_2^* \rightleftharpoons \text{RCl}^* + HgCl_2 \qquad k_e$$

Racemization in the benzhydryl system is difficult, requiring that the $R^+HgCl_3^-$ ion pairs become sufficiently loose to permit the $HgCl_3^-$ anion to migrate from one face of the cation to the other. This loss of rigidity in the ion pair is inevitably accompanied by randomization of the chlorine atoms on $HgCl_3^-$. It became of interest to determine how well the chlorine randomization process would compete with a more facile racemization process. *exo*-Norbornyl chloride (*exo*-RCl) seemed appropriate for this study since the ionization reaction proceeds with inevitable loss of optical activity.[4] We now wish to present the results of such an investigation.

In acetic acid at 75.0°, the solvolytic rate constants showed good second-order kinetics, first order in *exo*-RCl and first order in $HgCl_2$. The first-order solvolytic rate constants,[5] k_t, given by the product of the measured second-order rate constant and the initial $HgCl_2$ concentration show a linear dependence on $[HgCl_2]$ up to 0.058 *M*. These values are summarized in Table I. The increase in k_t due to added $HgCl_2$ is fit by a least-squares line with slope $4.05 \pm 0.37 \times 10^{-5}$ M^{-1} sec^{-1} and intercept 0.452×10^{-6} sec^{-1}. Similarly, the k_e values show a linear dependence on $[HgCl_2]$ where the slope of the least-squares line through the origin provides a second-order rate constant equal to $18.0 \pm 1.7 \times 10^{-5}$ M^{-1} sec^{-1}.

Table I. Summary of *k* Values for *exo*-Norbornyl Chloride

Salt, 10^2 *M*		10^6k, sec^{-1}		
[LiOAc]	[$HgCl_2$]	k_α[a]	k_t[b]	k_e[b]
		AcOH, 75.0°		
1.00			0.452 ± 0.013	
11.70		5.15 ± 0.17	0.604 ± 0.015	
11.7	1.10	9.58 ± 0.44		
2.33	1.17		1.02 ± 0.24	1.93 ± 0.12
2.15	2.20		1.40 ± 0.04	
2.33	2.40			4.16 ± 0.27
7.90	4.02	20.3 ± 0.10		
1.10	4.74			9.29 ± 0.96
1.94	4.82		2.72 ± 0.05	
5.15	5.40	26.1 ± 0.18		
2.08	5.75		3.27 ± 0.09	11.9 ± 1.2
		HCOOH, 25.0°		
1.43[c]			15.0 ± 0.2	
10.0[c]		60.7 ± 1.5[d]		
6.60[c]	1.91	112 ± 16[e]		
1.05[c]	2.28		47.8 ± 2.3	6.46 ± 0.17
1.28[c]	4.50		78.0 ± 2.2	12.6 ± 0.8

[a] 0.2–0.3 *M* RCl. [b] 0.01 *M* RCl. [c] Lithium formate. [d] 0.11 *M* RCl. [e] 0.058 *M* RCl.

Resolved *endo*-norbornyl alcohol[6] was converted to optically active *exo*-norbornyl chloride with phosphorus pentachloride in pyridine.[7] The rate of loss of optical activity for optically active *exo*-norbornyl chloride proceeds faster than the rates of acetolysis and exchange. The polarimetric rate constants (k_α) showed good first-order kinetics up to 70% loss of optical activity where the final solutions were 100 ± 0.7% racemic. The k_α values increase linearly with added

(1) Research supported by the National Science Foundation.
(2) S. Winstein, M. Hojo, and A. Ledwith, *Proc. Chem. Soc.*, 241 (1960).
(3) A. Diaz, I. L. Reich, and S. Winstein, *J. Amer. Chem. Soc.*, **92**, 7598 (1970).
(4) See the following and references therein: (a) S. Winstein, *et al.*, *ibid.*, 87, 376 (1965); (b) G. D. Sargent, *Quart. Rev., Chem. Soc.*, 20, 301 (1966).
(5) The kinetics and products of the reaction were followed by vpc analysis on a 1 m × $^1/_8$ in. column packed with 5% XF-1150 on 80–100 mesh Chromosorb W at 40° (15 psi).
(6) S. Winstein and D. Trifan, *J. Amer. Chem. Soc.*, **74**, 1147 (1952).
(7) E. Clippinger, Doctoral Dissertation, University of California, Los Angeles, 1955.