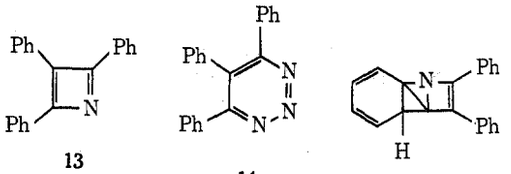
proposal might invoke a [$_{\pi}2 + {}_{\pi}2$] intramolecular cyclization to **15** which by suitable hydrogen shift and bond reorganization is transformed into **8**.[18]



13 14 15

Acknowledgment.—We wish to thank the National Science Foundation (GP-27956) for support.

(18) An analogous sequence has been proposed for the transformation of tetraphenylcyclobutadiene to 1,2,3-triphenylnaphthalene: G. Büchi, C. W. Perry, and E. W. Robb, *J. Org. Chem.*, **27**, 4106 (1962). Other mechanistic proposals may, of course, be offered for this reaction.

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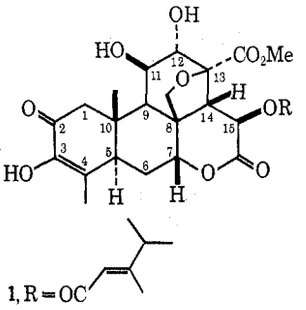
Bruceantin, a New Potent Antileukemic Simaroubolide from *Brucea antidysenterica*[1-3]

Summary: Bruceantin and bruceantarin, new antileukemic simaroubolides from *Brucea antidysenterica*, a plant used in Ethiopia in the treatment of cancer, are shown to have structures **1** and **2**, respectively.

Sir: *Brucea antidysenterica* Mill. is a simaroubaceous tree which is used in Ethiopia in the treatment of cancer.[4] In the course of a continuing search for tumor inhibitors from plant sources, we found that an alcoholic extract of *Brucea antidysenterica* Mill.[5] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) and against two standard animal tumor systems.[6] We report herein the isolation and structural elucidation of a new potent antileukemic simaroubolide tumor inhibitor, bruceantin (**1**),[7] and the companion simaroubolide bruceantarin (**2**), from *Brucea antidysenterica*.

Fractionation of the alcohol extract, guided by assay against KB and P-388, revealed that the inhibitory activity was concentrated, successively, in the chloroform layer of a chloroform–water partition, the methanol layer of a 10% aqueous methanol–petroleum ether partition, the methanol layer of a 20% aqueous methanol–carbon tetrachloride partition and, finally, in the chloroform layer of a chloroform–40% aqueous methanol partition. Column chromatography of the final chloroform-soluble material on SilicAR CC7 yielded two KB cytotoxic fractions (A and B) on elution with 1% methanol in chloroform. Continued elution with 2% methanol in chloroform gave a third cytotoxic fraction (C). Careful rechromatography of fraction A on SilicAR CC7 with 20% ether in benzene gave bruceantin (**1**, 0.01%): $C_{28}H_{36}O_{11}$; $[\alpha]^{25}D$ −27.7° (*c* 3.0, pyridine); uv max (EtOH) 280 nm (ε 6450) and 221 (14,100), uv max (EtOH + NaOH) 328 nm (ε 4260) and 221 (15,500); ir (KBr) 2.90, 5.76, 6.05, 6.13, 8.70, and 9.45 μ; mass spectrum *m/e* 548.222 (M^+, calcd 548.225), 438, 420, 402, 297, 151, 111.0819 (calcd, $C_7H_{11}O$, 111.0809); nmr ($CDCl_3$) τ 8.88 [6 H, d, J = 6.5 Hz, $CH(CH_3)_2$], 8.56 (3 H, s, 10-CH_3), 8.11 (3 H, br s, 4-CH_3), 7.82 [3 H, s, $CH{=}C(CH_3)$], 7.29 (1 H, br m, OH), 6.47 (1 H, br s, OH), 6.24 (3 H, s, OCH_3), 4.39 [1 H, br s, $O_2CCH{=}C(CH_3)$], 3.87 (1 H, br s, OH), and 3.79 (1 H, d, $J_{15,14}$ = 13 Hz, 15-H).



1, R = OC
2, R = OCC_6H_5
3, R = $OCCH_3$
4, R = H
5, R = $OCCH_2CH(CH_3)CH(CH_3)_2$

Rechromatography of fraction B on SilicAR CC7 with 30% ether in benzene gave bruceantarin (**2**, 0.002%): $C_{28}H_{30}O_{11}$; mp 182–185°; $[\alpha]^{25}D$ −20.7° (*c* 0.6, pyridine); uv max (EtOH) 278 nm (ε 7000) and 231 (10,500), uv max (EtOH + NaOH) 330 nm (ε 4480) and 230 (9030); ir (KBr) 2.9, 5.78, 6.03, 6.08, 6.12, 7.88, 8.70, 9.0, 9.45, and 13.8 μ; mass spectrum *m/e* 542 (M^+), 437, 420, 402, 297, 151, 105, and 77; nmr ($CDCl_3$) τ 8.63 (3 H, s, 10-CH_3), 8.20 (3 H, br s, 4-CH_3), 6.56 (3 H, s, OCH_3), 3.58 (1 H, d, $J_{15,14}$ = 13 Hz, 15-H), 2.60 (3 H, m, B_2X portion of A_2B_2X, *m* and *p*-benzoate protons), and 2.07 (2 H, d of d, A_2 part of A_2B_2X system, J_{AB} = 7.5, J_{AX} = 1.5 Hz, *o*-benzoate protons).

Rechromatography of fraction C on SilicAR CC7 using 2:1 ether in benzene gave the known bruceine B (**3**, 0.002%), characterized by comparison of its melting point, $[\alpha]D$, and ir, nmr, uv, and mass spectra with those previously reported.[8]

Bruceantin (**1**) and bruceantarin (**2**) gave a positive ferric chloride test, and displayed in their uv spectra the large bathochromic shift with alkali characteristic of diosphenols. In addition, acetylation of bruceantin (**1**) gave a triacetate which displayed neither the uv absorption at 280 nm nor the associated bathochromic shift. The mass spectra of **1** and **2** displayed as primary fragmentations peaks corresponding to a

(1) Tumor Inhibitors. LXXXII. Part LXXXI is ref 2.

(2) S. M. Kupchan and G. Tsou, *J. Org. Chem.*, in press.

(3) Supported by grants from the National Cancer Institute (CA-11718) and American Cancer Society (T-275 and IC-57H), and a contract with the National Cancer Institute (NIH-NCI-C-71-2099).

(4) J. L. Hartwell, *Lloydia*, **34**, 221 (1971).

(5) Stem bark was collected in Ethiopia in June 1971. Leaves and the wood of stems from Ethiopia also yielded active extracts. We thank Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., for supplying the plant material.

(6) Activity was noted against P-388 leukemia 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).

(7) Bruceantin showed significant antileukemic activity against P-388 lymphocytic leukemia over a 50–100-fold dosage range at the μg/kg level, and cytotoxicity (ED_{50}) against KB cell culture at 10^{-3} μg/ml. Bruceantarin showed only moderate activity against P-388, and the previously isolated[8] bruceine B showed only marginal activity against this system.

(8) J. Polonsky, Z. Baskevitch, A. Gaudemer, and B. C. Das, *Experientia*, **23**, 424 (1967).

loss of $C_7H_{10}O$ (*m/e* 438) and C_7H_5O (437) and base peaks corresponding to $C_7H_{11}O$ (111) and C_7H_5O (105), respectively. Except for the above-mentioned base peaks in the mass spectra of **1** and **2**, peaks in the region from *m/e* 438 to 69 were almost identical with those present in the mass spectrum of bruceine B (**3**). Inspection of the nmr spectra of bruceantin (**1**), bruceantarin (**2**), and bruceine B (**3**) revealed that all three displayed peaks corresponding to an angular methyl group in the region of τ 8.3–8.6, a vinyl methyl at 8.0–8.2, a methoxyl at 6.2–6.5, and a sharp one-proton doublet (J = 13 Hz) between 3.2 and 3.6 [assigned to H-15 in bruceine B (**3**)[8]]. The major differences between the nmr spectra of bruceantin (**1**) and bruceine B (**3**) were the additional signals for **1** of a six-proton doublet (J = 6.5 Hz) at τ 8.88, a vinyl methyl signal at 7.82, and a vinyl proton singlet at 4.39. These data and the presence of the base peak at *m/e* 111 in the mass spectrum supported formulation of bruceantin (**1**) as the 3,4-dimethylpent-2-enoic acid ester of bruceolide[8] (**4**). Hydrogenation of bruceantin (**1**) gave dihydrobruceantin (**5**): $C_{28}H_{38}O_{11}$; mp 137–140°; $[\alpha]^{25}D$ −64.5° (*c* 2.9, pyridine); mass spectrum *m/e* 550 (M^+), 438, 297, 151, and 113. That only the side-chain ester of **1** had been reduced was indicated by the uv spectrum, which still showed the diosphenol absorption and alkaline shift, and by the nmr spectrum, which showed no olefinic proton but a new three-proton doublet (J = 6.5 Hz) at τ 9.06. Mild alkaline hydrolysis of **5** gave bruceolide (**4**). In addition, alkaline hydrolysis of bruceantin (**1**) and esterification of the steam-distillable acid with diazoethane gave ethyl *trans*-3,4-dimethyl-2-pentenoate.[9] In the nmr spectrum of ethyl *cis*-3,4-dimethyl-2-pentenoate the vinyl methyl signal appeared at τ 8.25, whereas the corresponding peak for the trans isomer occurred at 7.90. The peak attributed to the ester vinyl methyl in **1** appeared at τ 7.82, indicative of trans stereochemistry in bruceantin (**1**).

The sharp one-proton doublet at τ 3.79 (J = 13 Hz) in the nmr spectrum of **1** indicated C-15 as the point of attachment of the ester side chain. The corresponding peak in the spectrum of dihydrobruceantin (**5**) appeared at τ 3.14 (J = 13 Hz) and in that of bruceine B (**3**) at τ 3.28 (J = 13 Hz).

In the nmr spectrum of bruceantarin (**2**), a complex A_2B_2X system centered at τ 2.3 was indicative of the presence of a benzoate group. In addition, the sharp one-proton doublet (J = 13 Hz) at τ 3.58 and the base peak at *m/e* 105 in the mass spectrum supported for bruceantarin (**2**) the C-15 benzoate ester structure. The postulated structure was confirmed by mild alkaline hydrolysis of bruceantarin (**2**) to benzoic acid and bruceolide (**4**).

The observed potent antileukemic activity of bruceantin confirms and extends an earlier report of antitumor activity of a simaroubolide.[10] The markedly higher potency of bruceantin (**1**),[7] compared with that of bruceantarin (**2**) and bruceine B (**3**), may be attributable to the role of the α,β-unsaturated ester.[11] Investigations are in progress to determine the significance of the unsaturated ester, the diosphenol, and of other structural features in relation to the tumor-inhibitory activity of bruceantin.

(9) M. J. Jorgenson and T. Leung, *J. Amer. Chem. Soc.*, **90**, 3769 (1968).
(10) M. E. Wall and M. Wani, *Int. Symp. Chem. Nat. Prod., 7th*, Abstracts E138, 614 (1970).
(11) *Cf.* S. M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970).

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Intramolecular Electrostatic Stabilization of an SNl Transition State

Summary: *o*-Carboxybenzal chloride hydrolyzes at about the same rate as the para isomer in water but 110 times as fast as in 60% aqueous dioxane.

Sir: The mechanism proposed for the hydrolytic action of lysozyme[1] involves at least two essential features. The first of these is the suggestion that Glu-35 acts as a general acid[2] effecting intracomplex protonation of the acetal linkage. It seems to be well established that a carboxyl group can function as an intermolecular general acid in acetal hydrolysis.[3] Intramolecular general acid catalysis has also recently been observed in the hydrolysis of 2-(*o*-carboxyphenoxy)tetrahydropyran in aqueous dioxane.[4] The second feature of the proposed enzymatic mechanism is that the ionized form of Asp-52 functions either as a nucleophile forming a glycosyl enzyme intermediate or electrostatically stabilizes the transition state leading to the oxocarbonium ion intermediate. In a very careful study Dunn and Bruice[5] have provided evidence that an ortho carboxylate ion can electrostatically stabilize the transition state in the A-1 cleavage of acetals and that this type of stabilization can provide substantial rate enhancements. To obtain additional information concerning the role of an ionized carboxyl group in stabilizing an ionic transition state uncomplicated by a proton-transfer step we have studied the hydrolysis of *o*- and *p*-carboxybenzal chlorides.

The mechanism of hydrolysis of benzal chlorides has been the subject of numerous reports.[6] It is clear that the mechanism involves rate-determining formation of a chlorocarbonium ion followed by a series of rapid steps leading to the product aldehyde (Scheme I). For example, ρ^+ calculated from published[6a] data is -5.2 ± 0.3. Also the rate is completely unaffected by external nucleophiles[6b,c,e] and the value

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