the methods. In both cases, the ion chromatographic method is more rapid and involves less sample manipulation than the traditional assays.

Cation Analysis—An ion chromatographic method, similar to the method reported for the determination of cations in ambient air aerosols (9), was successfully applied to the analysis of various penicillins (Figs. 3 and 4). The cation system, like the anion system, is independent of the nature of the counter-ion.

Linearity—Standards containing 5–15 μg/ml sodium and 10–40 μg/ml potassium gave linear responses by both peak height and peak area computation (Figs. 3 and 4). These ranges encompass the theoretical cation content for the antibiotic salts. The stoichiometric potassium level is 10.07% in penicillin V potassium (potassium phenoxymethyl penicillin) and 10.50% in penicillin G (potassium benzyl penicillin). Theoretical sodium levels in the sodium salts are 4.45% in carbenicillin indanyl sodium and 5.49% in carbenicillin sodium monohydrate. The sample concentrations (Table II) were chosen to give a similar response for both cations so that samples could be batched.

Reproducibility—Typical precisions of the ion chromatographic cation determination are shown in Table IV. These experiments include the complete replicate analysis of the same bulk lot.

Equivalency—No compendial requirement exists for the potassium content of the penicillins; however, both assays agreed well with the theoretical values.

The ion chromatographic sodium analysis was compared with sodium content determined by ashing six different antibiotic lots in the presence of sulfuric acid and measuring the remaining sodium sulfate gravimetrically. For carbenicillin sodium monohydrate, the relative difference was $-1.0 \pm 3.3\%$ (confidence interval: 1 *SD*). Carbenicillin indanyl sodium by ion chromatography was $3.9 \pm 4.3\%$ greater than the analysis by the residue on ignition method. Since both differences are smaller than the confidence interval, there was no apparent bias between methods.

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Antitumor Agents XLV: Bisbrusatolyl and Brusatolyl Esters and Related Compounds as Novel Potent Antileukemic Agents

KUO-HSIUNG LEE *x, MASAYOSHI OKANO *, IRIS H. HALL *, DAVID A. BRENT ‡, and BERND SOLTMANN ‡

Received April 6, 1981, from the **Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514* and the ‡Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC 27709. Accepted for publication July 15, 1981.

Abstract □ A series of new bisbrusatolyl and brusatolyl esters and related compounds were synthesized and tested for *in vivo* antileukemic activity against a quassinoid sensitive strain of P-388 lymphocytic leukemia in BDF_1 mice. The bisbrusatolyl malonate, succinate, glutarate, adipate, and sebacate were as active or more active than brusatol. The C-3 esters of brusatol and bruceantin were also found to be as active or more active than brusatol or bruceantin in general. The free hydroxyl groups at C-11 and C-12 as well as the enone double bond in ring A of both bisbrusatolyl and brusatolyl esters are required for antileukemic activity. The presence of a double bond in the ester side chain contributes to the enhanced activity of these esters.

Keyphrases □ Brusatol—bisbrusatolyl and bisbrusatolyl esters, synthesis, potential antileukemic agents □ Antitumor agents—potential, bisbrusatolyl and bisbrusatolyl esters, synthesis □ Antileukemic agents—potential, bisbrusatolyl and bisbrusatolyl esters, synthesis, tested against P-388 lymphocytic leukemia

The structural requirements for antineoplastic activity (particularly in the P-388 mouse lymphocytic leukemic system) of quassinoids bruceantin (I), holacanthone, glaucarubolone, 6α-senecioyloxychaparrinone, and related compounds have recently been reviewed (1–6). It was concluded that the Δ^3-2-oxo moiety in ring A, the lactone moiety in ring D, the ester groups at either C-6 or C-15, the methyleneoxy bridge, and the hydroxyl moieties at either C-1 or C-3, and at C-12 are required for biological activity.

The isolation of novel antileukemic glycosides bruceoside-A and bruceoside-B, as well as their subsequent hydrolysis product brusatol (II) (7, 8) provided the opportunity for developing brusatol related compounds into future clinically active anticancer agents. Brusatol is structurally identical to bruceantin (I) [currently in the Phase II clinical trial as an anticancer agent by the National Cancer Institute (9)] except for a slight difference in the C-15 ester side chain. The C-15 ester moiety in I is important for its potent antileukemic activity and probably serves as a carrier group in processes such as membrane transport of the drug into intact cells or complex formation as previously suggested (2, 6, 10). The importance of the ester group which contributes to the enhanced antileukemic activity is also seen in other naturally oc-

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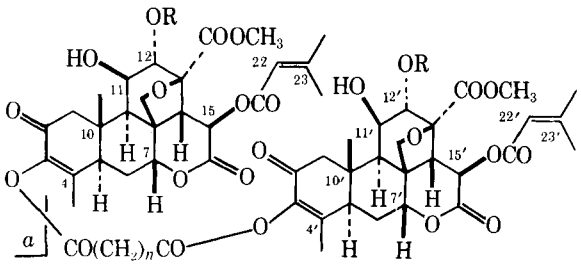
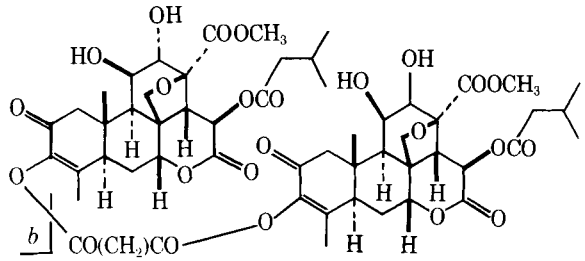
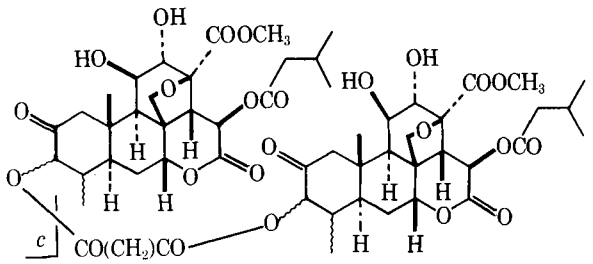


III: n = 1; R = H
IV: n = 2; R = H
V: n = 3; R = H
VI: n = 4; R = H
VII: n = 8; R = H
VIII: n = 2; R = $COCH_3$
IX: n = 3; R = $COCH_3$
X: n = 4; R = $COCH_3$



XI: n = 1
XII: n = 3



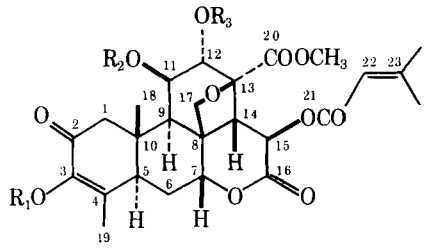
XIII

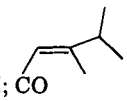
curring antitumor agents (11).

In view of the overall structural requirements for the antileukemic activity of I, any major degradation of the intact I molecule might thus lead to the less active or inactive compounds. However, a minor modification by combining two intact active quassinoids *via* a diester linkage (such as I or II) might yield highly active antileukemic agents with reduced toxicity as observed with bishelenalinyl malonate and related esters (1, 11). An initial examination of the antileukemic activity of bisbrusatolyl malonate (III) and bisbrusatolyl succinate (IV) against a quassinoid-sensitive strain of P-388 lymphocytic leukemia indicated that III and IV were more potent and less toxic than brusatol (11). For example, the optimal T/C % value for III was 272 at 0.6 mg/kg, while for II it was 158 at 0.125 mg/kg. The synthesis and antileukemic activity of bisbrusatolyl and brusatolyl esters and related compounds are now reported.

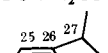
CHEMISTRY

Bisbrusatolyl esters III–VII were prepared by reacting brusatol II with malonic dichloride, succinic dichloride, glutaric dichloride, adipic dichloride, and sebacic dichloride, respectively, in pyridine–benzene or pyridine–chloroform mixed solvent at room temperature. The monoacetates (VIII–X) of the bisbrusatolyl esters IV–VI were prepared by acetylation using acetic anhydride–pyridine and worked up as usual. Catalytic hydrogenation of the C-15 ester side chain of III and V with palladium-on-carbon in ethanol gave rise to the corresponding bis-dihydro derivatives XI and XII, respectively. The bistetrahydrobrusatolyl succinate (XIII) was prepared by esterification of tetrahydrobrusatol (XXXV), obtained by catalytic hydrogenation of II, with succinic dichloride.

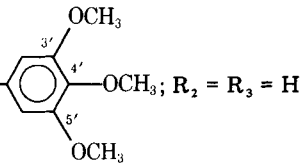


I: R_1 = R_2 = R_3 = H; CO  replaces CO  (bruceantin)
II: R_1 = R_2 = R_3 = H (brusatol)

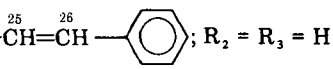
XIV: R_1 = CO ; R_2 = R_3 = H
XV: R_1 = $CO(CH_2)_3CH_3$; R_2 = R_3 = H
XVI: R_1 = $COCH_2CH_2COOCH_2CH_3$; R_2 = R_3 = H



XVII: R_1 = CO ; R_2 = R_3 = H

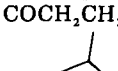


XVIII: R_1 = CO–; R_2 = R_3 = H

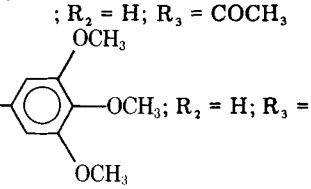


XIX: R_1 = CO–CH=CH–; R_2 = R_3 = H

XX: R_1 = CO ; R_2 = H; R_3 = $COCH_3$
XXI: R_1 = $CO(CH_2)_3CH_3$; R_2 = H; R_3 = $COCH_3$
XXII: R_1 = $COCH_2CH_2COOCH_2CH_3$; R_2 = H; R_3 = $COCH_3$



XXIII: R_1 = CO ; R_2 = H; R_3 = $COCH_3$



XXIV: R_1 = CO–; R_2 = H; R_3 = $COCH_3$

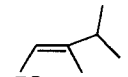
XXV: R_1 = CO ; R_2 = R_3 = $COCH_3$
XXVI: R_1 = $CO(CH_2)_3CH_3$; R_2 = R_3 = $COCH_3$
XXVII: R_1 = $COCH_2CH_2COOCH_2CH_3$; R_2 = R_3 = $COCH_3$

Encouraged by the potent antileukemic activity and reduced toxicity demonstrated by the bisbrusatolyl esters (III–VII) resulting from esterification of the C-3 hydroxyl group of the diosphenol ring A, it was decided to further investigate the C-3 esters of brusatol (II). Thus, various C-3 esters, such as senecioate (XIV), valerate (XV), ethyl succinate (XVI), 3,4-dimethyl-2-pentenoate (XVII), 3′,4′,5′-trimethoxybenzoate (XVIII), and cinnamate (XIX), were prepared by esterification of II with a corresponding acyl chloride in pyridine–benzene or pyridine–chloroform mixed solvent at room temperature. Esterification of II with cinnamoyl chloride under these conditions yielded a C-3, C-11 dicinnamate (XXXI) in addition to the C-3 cinnamate (XIX). Esterification of bruceantin (I)

in an analogous manner afforded its C-3, 3,4-dimethyl-2-pentenoate ester (XXXIII).

Esterification of C-3, C-11, and C-12 hydroxyl groups is greatly affected by the reaction temperature and time. The C-11 hydroxyl group is sterically hindered and resistant to esterification under mild reaction conditions like those for the selective esterification of C-3. In general, it is required to heat the reaction mixture up to ~60° for esterification of C-11 and C-12 hydroxyl groups other than those involving acetylation. However, for esterification of the C-3 hydroxyl group, the reaction may be run at room temperature. The introduction of a C-3 ester was confirmed by examining the UV spectrum which should show the disappearance of a diosphenol chromophore at λ_{max} 278 nm. Confirmation of the presence of ester moieties at C-11 and C-12 was achieved by observing the downfield shift of H-11 and H-12 resulting from such esterification.

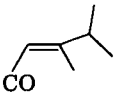
The mono- (XX–XXIV), di- (XXV–XXIX), and tri- (XXX) acetates of II were prepared by acetylation of the corresponding esters XIV–XIX with acetic anhydride in pyridine. The tri-ester (XXXII) was prepared by treating II with 3,4-dimethyl-2-pentenoyl chloride in dry benzene under reflux for 24 hr. The dihydro- (XXXIV) and tetrahydro- (XXXV) brusatol were prepared by catalytic hydrogenation of II with palladium-on-carbon. Sodium borohydride reduction of XXXV gave rise to the lactol (XXXVI). Spectral data (Table I) of all compounds (II–XXXIV) were in accord with the assigned structures.

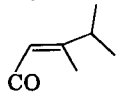
XXVIII: R_1 =  ; $R_2 = R_3 = COCH_3$

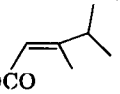
XXIX: R_1 = CO—CH=CH—  ; $R_2 = R_3 = COCH_3$

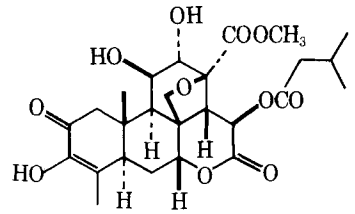
XXX: $R_1 = R_2 = R_3 = COCH_3$

XXXI: $R_1 = R_2$ = CO—CH=CH—  ; $R_3 = H$

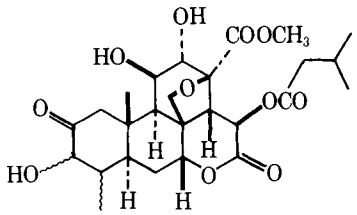
XXXII: $R_1 = R_2 = R_3$ = 

XXXIII: R_1 =  ; $R_2 = R_3 = H$;

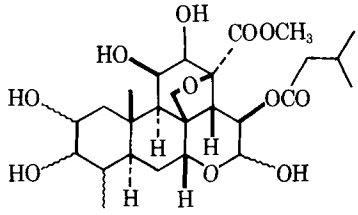
C_{15}-OCO  replaces C_{15}-OCO 



XXXIV



XXXV



XXXVI

DISCUSSION

The preliminary evaluations of the antileukemic activity of compounds II–XXXVI were carried out in a quassinoid sensitive strain of P-388 lymphocytic leukemia in BDF_1 mice which were described previously (11). A comparison of the T/C % values for the antileukemic activity of the compounds listed in Table II disclosed that the bisbrusatolyl esters (III–VII) possessed equal or more potent activity than brusatol at 0.6 mg/kg (compare T/C% of 272, 217, 176, 176, and 143 for III, IV, V, VI, and VII, respectively, to 149 for II). Compound III followed a dose response curve and demonstrated potent antileukemic activity (T/C = 213, 272, and 215% at doses of 1.0 mg, 0.6 mg, and 0.3 mg/kg/day, respectively). At comparable dose levels, brusatol was either much less active or more toxic. Monoacetylation of the 12- and 12′-hydroxyl groups of the bisesters (IV–VI) resulted in less active or inactive compounds (VIII–X), suggesting that the presence of such hydroxyl groups at the 12- and 12′-positions are required for antileukemic activity. Reduction of the senecioate ester double bonds of III and V afforded the corresponding bisdihydro derivatives XI and XII, in which XI (T/C = 132%) and XII (T/C = 147%) were less active than III and V. Saturation of the diosphenol double bond, as in the case of bistetrahydrobrusatolyl succinate (XIII), gave rise to an inactive compound.

Compounds XIV–XIX were various C-3 esters of brusatol (II). Again it was observed that the esters were more active or as active as II at 0.6 mg/kg/day (compare T/C of 185–130% of XIV–XIX to 149% of II). Similarly, the C-3, 3,4-dimethyl-2-pentenoate ester of bruceantin (*i.e.*, XXXIII), demonstrated a T/C of 194% at the same testing dose. Acetylation of compounds XIV–XIX either at the 12-hydroxyl moiety or at both the 11- and 12-hydroxyl groups yielded compounds XX–XXIV and XXV–XXIX, respectively, which showed no activity. The 11,12-diacetates of 3-cinnamoyl brusatol (XXIX) and of 3-acetyl brusatol (XXX), as well as the 3,11,12-tri-3,4-dimethyl-2-pentenoyl brusatol (XXXII) were all found to be inactive. However, the 3,11-dicinnamoyl brusatol (XXXI) in which the 12-hydroxyl group was free was found to give a T/C of 133% indicating the importance of a free hydroxyl group at position 12 for significant antileukemic activity. Reduction of the ester double bond of II yielded dihydrobrusatol (XXXIV), which did not decrease the activity. However, destruction of the enone system in ring A gave rise to XXXV which was much less active. Further reduction of the carbonyl at C-2 and C-16 yielded the inactive lactol (XXXVI).

EXPERIMENTAL[1]

Brusatol (II)—A solution of bruceoside-A (7, 8) (101 g, 0.148 mole) in 3 *N* sulfuric acid–methanol (1:1, 2000 ml) was stirred at 65° for 20 hr. The hydrolyzed product was extracted with chloroform to give a brown resinous substance (76.9 g). Column chromatography of this substance on silica gel yielded crude brusatol (67.7 g, 86%), which was further recrystallized from acetone to give II as colorless crystals: mp 274–277°.

[1] Unless otherwise specified, melting points were determined on a Thomas–Hoover melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. PMR spectra were measured with a Varian XL-100 instrument (tetramethylsilane). Electron impact mass spectra were determined on an AEI MS-902 instrument at 70 eV using a direct inlet system. The field desorption mass spectra were obtained on a Varian MAT 731 mass spectrometer interfaced to a VG 2200 Datasystem (13). All spectra were run in a cyclic scanning mode with a steady increase of the emitter current from 0–50 mA. The sample was coated on the field desorption wire using the dipping technique. The solution used for dipping contained 5–10 μg of sample/μl of acetone. All samples desorbed at emitter currents between 22 and 26 mA. Compounds XIV–XXXII gave intense $[M]^+$ and/or $[M + H]^+$ ions. The esters of the dicarboxylic acids gave no molecular ions. Rather, these compounds were protonated on the ester oxygen and gave an ion due to simple cleavage, *e.g.* $[a + H]^+$. Some showed only ions which were produced by the net loss of water from this simple cleavage ion. Silica gel for column chromatography refers to Mallinckrodt Silica AR CC7-Special; silica gel for preparative TLC refers to Merck precoated silica gel GF-254 (0.25 mm, 5 × 20 cm) developed with suitable solvent systems and visualized by spraying with 1% cesium sulfate—10% sulfuric acid solution followed by heating or by use of an UV lamp. Pyridine and chloroform used as solvents for reactions were predried on molecular sieve and anhydrous calcium chloride, respectively. Elemental analyses were performed by Integral Microlab, Inc., Raleigh, N.C. All compounds (II–XXXVI) reported gave either satisfactory elemental or mass spectral (high resolution electron impact or field desorption) analyses (Table III). Also, all new compounds have been rigorously purified to homogeneity by TLC in at least three solvent systems. High resolution mass spectrometry was used in lieu of combustion elemental analysis in many cases due to the limited supply of natural brusatol.

Table I—NMR Spectra of Bisbrusatolyl and Brusatolyl Esters and Related Compounds[a]

Compd	—CH_2CO—	H-15 (15′)	H-22 (22′)	H-7 (7′)	H-11 (11′)	H-12 (12′)	$COOCH_3$	CH_3-23 (23′)	CH_3-4 (4′)	CH_3-10 (10′)	H-17 (17′)	H-1 (1′), H-6 (6′)	H-14 (14′)	Misc.
II		6.26 d (13)	5.64 m	4.80 m	4.25 m	4.22 m	3.80 s	2.20 d (1.5) 1.93 d (1.5)	1.84 d (2)	1.39 s	4.76 d (8) 3.80 d (8)	2.98 d (16) 2.40 d (16)	3.12 d (13)	
III	3.79 bs[b]	6.26 d (13)	5.66 m	4.83 m	4.30 m	4.22 m	3.79 bs[b]	2.18 d (1.5) 1.92 d (1.5)	1.84 s	1.48 s	4.76 d (8) 3.82 d (8)	3.00 d (16) 2.44 d (16)	3.16 d (13)	
IV	2.95 t (6)	6.24 d (13)	5.66 m	4.83 m	4.27 m	4.21 m	3.78 s	2.18 bs 1.94 bs	1.78 bs	1.47 bs	4.76 d (8) 3.78 d (8)	2.95[b] 2.44 d (16)	3.17 d (13)	
V	2.71 (t-like)	6.24 d (13)	5.67 m	4.85 m	4.30 m	4.23 m	3.78 s	2.19 bs 1.93 bs	1.79 bs	1.48 s	4.77 d (8) 3.80 d (8)	2.97 d (16) 2.42 d (16)	3.17 d (13)	
VI	2.60 (t-like)	6.21 d (13)	5.65 m	4.83 m	4.26 m	4.21 m	3.78 s	2.19 bs 1.94 bs	1.80 bs	1.49 s	4.75 d (8) 3.80 d (8)	2.96 d (16) 2.42 d (16)	3.18 d (13)	
VII	2.52 t (6)	6.24 d (13)	5.65 m	4.83 m	4.28 m	4.22 m	3.79 s	2.18 bs 1.92 bs	1.78 bs	1.48 s	4.76 d (8) 3.80 d (8)	2.98 d (16) 2.40 d (16)	3.18 d (13)	1.35 m (Methylene)
VIII	2.96 (t-like)	6.01 d (13)	5.64 m	4.82 m	4.12 m	5.31 m	3.76 s	2.10 bs 1.95 bs	1.80 bs	1.48 bs	4.78 d (8) 3.80 d (8)	2.94 d (16) 2.40 d (16)	3.30 d (13)	2.02 s ($OCOCH_3$)
IX	2.70 t (6)	6.04 d (13)	5.65 m	4.85 m	4.15 d (5)	5.33 m	3.75 s	2.18 bs 1.93 bs	1.79 bs	1.47 s	4.80 d (8) 3.80 d (8)	2.92 d (16) 2.38 d (16)	3.30 d (13)	2.00 s ($OCOCH_3$)
X	2.60 (t-like)	6.06 d (13)	5.64 m	4.82 m	4.14 m	5.32 m	3.76 bs	2.20 bs 1.94 bs	1.81 bs	1.50 s	4.78 d (8) 3.82 d (8)	2.94 d (16) 2.40 d (16)	3.30 d (13)	2.03 s ($OCOCH_3$)
XI	3.85[b]	6.33 d (13)		4.79 m	4.26 m	4.21 m	3.85[b]	0.94 (6)	1.83 bs	1.47 bs	4.76 d (8) 3.85[b]	2.98 d (16) 2.44 d (16)	3.08 d (13)	
XII	2.70 t (6)	6.30 d (13)		4.78 m	4.21 m	4.21 m	3.83 s[b]	0.97 (6)	1.79 bs	1.47 s	4.74 d (8) 3.83 m[b]	2.80 d (16) 2.38 d (16)	3.08 d (13)	
XIII	3.50 m	6.33 d (13)		4.70 m	4.21 m	4.21 m	3.84 bs	0.99 (6)	0.99 d (6)	1.45 s	4.66 d (8) 3.76 d (8)	2.76 d (16) 2.18 d (16)	3.08 d (13)	
XIV		6.23 d (13)	5.66 m	4.84 m	4.30 m	4.24 m	3.78 s	2.20 bs 1.93 bs	1.80 s	1.52 s	4.76 d (8) 3.78 d (8)	2.99 d (16) 2.44 d (16)	3.20 d (13)	5.91 m (H-25); 2.20 bs, 1.97 bs (CH_3-26)
XV		6.22 d (13)	5.64 m	4.82 m	4.27 m	4.21 m	3.77 s	2.17 bs 1.92 bs	1.78 s	1.48 s	4.74 d (8) 3.78 d (8)	2.96 d (16) 2.54 d (16)	3.17 d (13)	0.93 t (7) (terminal CH_3)
XVI		6.22 d (13)	5.65 m	4.83 m	4.27 m	4.16 m	3.78 s	2.19 bs 1.93 bs	1.81 s	1.47 s	4.74 d (8) 3.78 d (8)	2.96 d (16) 2.42 d (16)	3.18 d (13)	4.16 q (7), 1.26 t (7) (ethyl ester)
XVII		6.22 d (13)	5.65 m	4.83 m	4.28 m	4.22 m	3.77 s	2.18 bs 1.92 d (1.5)	1.79 s	1.51 s	4.75 d (8) 3.78 d (8)	2.98 d (16) 2.42 d (16)	3.18 d (13)	5.90 m (H-25); 2.16 bs (CH_3-26) 1.10 d (7) (CH_3-27)
XVIII		6.29 d (13)	5.66 m	4.84 m	4.30 d (5)	4.23 m	3.79 s	2.19 d (1.5) 1.93 d (1.5)	1.85 s	1.58 s	4.79 d (8) 3.93[b]	3.04 d (16) 2.46 d (16)	3.17 d (13)	7.42 s (phenyl) 3.93 s (OCH_3)
XIX		6.28 d (13)	5.66 m	4.82 m	4.30 m	4.23 m	3.80 s	2.21 d (1.5) 1.94 d (1.5)	1.86 s	1.56 s	4.78 d (8) 3.80 d (8)	3.03 d (16) 2.46 d (16)	3.18 d (13)	7.55–7.45 m (phenyl); 6.61 d (16) and 7.85 d (16) (H-25, H-26)
XX		6.02 d (13)	5.65 m	4.84 m	4.14 d (5)	5.32 m	3.74 s	2.19 bs 1.93 d (1.5)	1.80 s	1.52 s	4.80 d (8) 3.80 d (8)	2.96 d (16) 2.38 d (16)	3.30 d (13)	5.91 m (H-25); 2.19 bs, 1.96 bs (CH_3-23 and -26); 2.01 s ($OCOCH_3$)
XXI		6.00 d (13)	5.62 m	4.81 m	4.12 d (5)	5.30 m	3.73 s	2.17 bs 1.92 bs	1.77 bs	1.47 s	4.77 d (8) 3.78 d (8)	2.94 d (16) 2.36 d (16)	3.29 d (13)	0.92 t (7) (terminal CH_3) 2.00 s ($OCOCH_3$)
XXII		5.98 d (13)	5.62 m	4.80 m	4.10[b]	5.29 m	3.74 s	2.18 bs 1.93 d (1.5)	1.80 bs	1.47 s	4.76 d (8) 3.78 d (8)	2.93 d (16) 2.34 d (16)	3.28 d (13)	4.14 q (7) 1.25 t (7) (ethyl ester); 2.01 s ($OCOCH_3$)
XXIII		6.04 d (13)	5.65 m	4.84 m	4.14 d (5)	5.32 m	3.76 s	2.19 bs 1.94 bs	1.81 s	1.53 s	4.80 d (8) 3.80 d (8)	2.96 d (16) 2.39 d (16)	3.31 d (13)	5.92 m (H-25); 2.16 bs (CH_3-26); 2.03 s ($OCOCH_3$)
XXIV		6.05 d (13)	5.65 m	4.85 m	4.16 m	5.34 m	3.76 s	2.18 bs 1.92 bs	1.84 s	1.56 s	4.80 d (8) 3.76 s (8)	3.00 d (16) 2.42 d (16)	3.15 d (13)	7.42 s (phenyl), 3.92 bs (OCH_3) 2.02 s ($OCOCH_3$)

continued on next page

Table I—*Continued*

Compd	—CH_2CO—	H-15 (15′)	H-22 (22′)	H-7 (7′)	H-11 (11′)	H-12 (12′)	$COOCH_3$	CH_3-23 (23′)	CH_3-4 (4′)	CH_3-10 (10′)	H-17 (17′)	H-1 (1′), H-6 (6′)	H-14 (14′)	Misc.
XXV		6.10 d (13)	5.65 m	4.87 m	5.24 d (5)	5.34 m	3.75 s	2.19 bs 1.94 d (1.5)	1.81 d (2)	1.36 s	4.78 d (8) 3.85 d (8)	3.10 d (16) 2.52 d (16)	3.28 d (13)	5.91 m (H-25); 2.19 bs, 1.97 bs (CH_3-23 and -26) 2.04 s, 2.12 s ($OCOCH_3$)
XXVI		6.07 d (13)	5.62 m	4.84 m	5.22 d (5)	5.31 m	3.71 s	2.17 d (1.5) 1.92 d (1.5)	1.78 d (2)	1.31 s	4.75 d (8) 3.83 d (8)	3.08 d (16) 2.38 d (16)	3.26 d (13)	0.93 t (7) (terminal CH_3), 2.02 s ($OCOCH_3$) 2.10 s ($OCOCH_3$)
XXVII		6.06 d (13)	5.62 m	4.83 m	5.21 d (5)	5.31 m	3.72 s	2.19 d (1.5) 1.92 d (1.5)	1.81 d (2)	1.30 s	4.74 d (8) 3.82 d (8)	2.92 d (16) 2.48 d (16)	3.26 d (13)	4.15 q (7) 1.25 t (7) (ethyl ester); 2.03 s, 2.11 s ($OCOCH_3$)
XXVIII		6.10 d (13)	5.65 m	4.88 m	5.25 d (5)	5.35 m	3.74 s	2.20 bs 1.94 d (1.5)	1.82 s	1.37 s	4.79 d (8) 3.86 d (8)	3.10 d (16) 2.52 d (16)	3.29 d (13)	5.92 m (H-25); 2.16 bs (CH_3-26); 2.05 s, 2.13 s ($OCOCH_3$)
XXIX		6.13 d (13)	5.66 m	4.89 m	5.27 d (5)	5.36 m	3.74 s	2.21 d (1.5) 1.94 d (1.5)	1.86 s	1.37 s	4.80 d (8) 3.87 d (8)	3.14 d (16) 2.43 d (16)	3.30 d (13)	7.54–7.46 m (phenyl); 6.61 d (16), 7.85 d (16) (H-25, H-26); 2.05 s, 2.16 s ($OCOCH_3$)
XXXI		6.26 d (13)	5.68 m	4.92 m	5.53 d (5)	4.23 m	3.77 s	2.21 bs 1.94 bs	1.85 s	1.43 s	4.88 d (8) 3.90 d (8)	3.14 d (16) 2.45 d (16)	3.29 d (13)	7.53–7.44 m (phenyl); 6.59 d (16), 7.82 d (16) (H-25, H-26 of C_3); 6.41 d (16) 7.70 d (16) (H-25, H-26 of C_{11})
XXXII		6.14 d (13)	5.70 m	4.86 m	5.31 d (5)	5.30 m	3.69 m	2.18 bs 1.90 bs	1.80 bs	1.32 s	4.82 d (8) 3.84 d (8)	2.92 d (16) 2.36 d (16)	3.30 d (13)	5.87 m (H-25); 2.17 bs (CH_3-26 of C_3); 1.10 d (7) (CH_3-27 of C_3) 2.14 bs, 2.13 bs (CH_3-26 of C_{11} and C_{12}); 1.08 d (7), 1.06 d (7) (CH_3-27 of C_{11} and C_{12})

[a] Run in $CDCl_3$ at 100 MHz, and values are parts per million. Multiplicities are indicated by the usual symbols: d, doublet; t, triplet; m, multiple whose center is given; bs, slightly broadened singlet. Figures in parentheses are coupling constants in hertz. [b] Overlapped signals.

The identity of II as brusatol was confirmed by a direct comparison (mixed melting point, TLC, and IR, PMR, and mass spectra) with an authentic sample.

Synthesis of III–VII—3,3-Bisbrusatolyl malonate (III), succinate (IV), glutarate (V), adipate (VI), and sebacate (VII) were synthesized. Esterification was accomplished by treating II (~0.2 mmole) in dry pyridine (2 ml) with a solution of the corresponding malonyl (0.348 mmole), succinyl (0.738 mmole), glutaryl (0.74 mmole), adipyl (0.74 mmole), and sebacyl (0.215 mmole) dichlorides in dry chloroform (2 ml) and cooling with ice. After the mixture was stirred at room temperature for 19, 19, 40, 20, and 20 hr, respectively, it was shaken with dilute sulfuric acid to remove pyridine. The product was extracted with chloroform and purified by preparative TLC (chloroform–acetone 1:1).

III: pale blue crystals (19% yield); mp 191–193°; UV (ethyl alcohol) 221 nm (log ϵ 4.38); IR (potassium bromide) 3450 (OH), 1730, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1645 (C=C) cm^{-1}.

IV: colorless crystals (61% yield); mp 248–250°; UV (ethyl alcohol) 221 nm (log ϵ 4.45); IR (potassium bromide) 3480 (OH), 1730, 1710 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1645 (C=C) cm^{-1}.

V: colorless crystals (39% yield); mp 166–168°; UV (ethyl alcohol) 221 nm (log ϵ 4.45); IR (potassium bromide) 3440 (OH), 1740, 1715 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

VI: colorless crystals (53% yield); mp 239–241°; IR (potassium bromide) 3480 (OH), 1725 (ester and lactone CO), 1675 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

Table II—Antileukemic Activity of Bisbrusatolyl and Brusatolyl Esters and Related Compounds against P-388 Lymphocytic Leukemia Cell Growth in BDF_1 Mice

Compound	Dose, mg/kg/day intraperitoneally	Average Days Survived of Treated/Control	T/C, %[a]
II	0.6	14.2/9.5	149
	0.3	14.3/9.5	150
	0.25	14.5/9.5	153
	0.125	15.0/9.5	158
	0.100	12.8/9.5	134
III	1.0	20.2/9.5	213
	0.6	25.8/9.5	272
	0.3	20.4/9.5	215
	0.25	15.5/9.5	163
	0.125	11.0/9.5	116
	0.100	11.0/9.5	116
IV	0.6	20.6/9.5	217
V	0.6	16.7/9.5	176
VI	0.6	16.8/9.5	176
VII	0.6	13.6/9.5	143
VIII	0.6	11.2/9.5	118
IX	0.6	11.6/9.5	122
X	0.6	9.21/9.5	97
XI	0.6	12.5/9.5	132
XII	0.6	13.9/9.5	147
XIII	0.6	10.7/9.5	113
XIV	0.6	17.6/9.5	185
XV	0.6	15.7/9.5	166
XVI	0.6	15.3/9.5	161
XVII	0.6	14.2/9.5	149
XVIII	0.6	12.3/9.5	130
XIX	0.6	13.0/9.5	137
XX	0.6	9.8/9.5	103
XXI	0.6	9.9/9.5	104
XXII	0.6	11.4/9.5	120
XXIII	0.6	9.9/9.5	105
XXIV	0.6	10.6/9.5	112
XXV	0.6	9.8/9.5	103
XXVI	0.6	10.3/9.5	109
XXVII	0.6	10.4/9.5	109
XXVIII	0.6	10.2/9.5	107
XXIX	0.6	9.0/9.5	95
XXX	0.6	9.7/9.5	102
XXXI	0.6	11.2/9.5	118
XXXII	0.6	12.6/9.5	133
XXXIII	0.6	18.4/9.5	194
XXXIV	0.6	14.2/9.5	150
XXXV	0.6	11.4/9.5	120
XXXVI	0.6	10.2/9.5	107
5-Fluorouracil	12.5	15.7/9.5	166

[a] A compound is active if it exhibits a T/C $\geq$ 125% (12).

VII: colorless crystals (79% yield); mp 173–175°; UV (ethyl alcohol) 220 nm (log ϵ 4.60); IR (potassium bromide) 3450 (OH), 1730, 1715 (ester and lactone CO), and 1650 (C=C) cm^{-1}.

General Method for the Synthesis VIII–X—12,12′-Diacetoxy-3,3′-bisbrusatolyl succinate (VIII), glutarate (IX), and adipate (X) were synthesized. These 12,12′-diacetates were prepared by acetylation of the corresponding IV–VI with dry pyridine–acetic anhydride (1:1) for 18–24 hr at room temperature with stirring followed by workup with preparative TLC (chloroform–acetone 1:1) purification.

VIII: colorless crystals (42% yield); mp 204–206°; IR (potassium bromide) 3480 (OH), 1735 (ester and lactone CO), 1685 (α,β-unsaturated CO), 1640 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

IX: colorless crystals (85% yield); mp 213–215°; IR (potassium bromide) 3440 (OH), 1740 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

X: colorless crystals (44% yield); mp 248–250°; IR (potassium bromide) 3490 (OH), 1730 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), and 1215 ($OCOCH_3$) cm^{-1}.

General Method for the Synthesis of XI and XII—3,3′-Bis-22,23-dihydrobrusatolyl malonate (XI) and glutarate (XII) were synthesized. A solution of III (36.7 mg, 0.0331 mmole) and V (30 mg, 0.0264 mmole) in absolute ethanol (3 and 2 ml, respectively) was hydrogenated in the presence of prereduced 10% palladium-on-charcoal (48 and 47 mg, respectively and at 65 and 70° for 8 and 48 hr, respectively). Compounds XI and XII were obtained by eluting the reaction mixture with ethanol through a column containing anhydrous magnesium sulfate.

XI: colorless crystals (48% yield); mp 208–210°; IR (potassium bromide) 3480 (OH), 1740 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

XII: colorless crystals (83% yield); mp 223–225°; IR (potassium bromide) 3420 (OH), 1730, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

Synthesis of XIII—3,3′-Bis-3,4,22,23-tetrahydrobrusatolyl succinate (XIII) was synthesized. A solution of IV (32.65 mg, 0.0291 mmole) in ethanol (2 ml) was hydrogenated in the presence of prereduced 10% palladium-on-charcoal (58 mg) at 60° for 44 hr until the disappearance of UV absorption at λ_{max} ~220 nm. The product was purified directly by preparative TLC (chloroform–acetone 1:1) to yield XIII as colorless crystals (21.65 mg, 66%): mp 280° (dec.); IR (potassium bromide) 3430 (OH), 1735, and 1725 (ester and lactone CO) cm^{-1}.

General Method for the Synthesis of XIV–XIX and XXXI—Brusatol-3-yl senecioate (XIV), valerate (XV), ethyl succinate (XVI), 3,4-dimethyl-2-pentenoate (XVII), 3′,4′,5′-trimethoxybenzoate (XVIII), cinnamoate (XIX), and brusatol-3,11-diyl dicinnamoate (XXXI) were synthesized. These esters were prepared by treatment of II (105 mg, ~0.2 mmole) in dry pyridine (2 ml) with a solution of the corresponding senecioyl (171 mg, 1.44 mmoles), valeryl (170 mg, 1.41 mmoles), ethyl succinyl (64 mg, 0.43 mmole), 3,4-dimethyl-2-pentenoyl (106.4 mg, 0.726 mmole), 3′,4′,5′-trimethoxybenzoyl (154 mg, 0.67 mmole, in 10 ml of dry benzene), and cinnamoyl (166.6 mg, 1.00 mmole) chlorides in dry chloroform (2 ml) under cooling with ice. After the mixture was stirred at room temperature for 2, 24, 20, 28, 50, and 24 hr, respectively, and worked up in a manner analogous to the synthesis of III–VII, it was subjected to preparative TLC using chloroform–acetone 10:1 for XIV, XVII, XIX, and XXXI, and chloroform–acetone 1:1 for XV, XVI, and XVIII.

XIV: colorless crystals (83 mg, 66% yield); mp 143–145°; IR (potassium bromide) 3400 (OH), 1725, 1710 (ester and lactone CO), 1675 (α,β-unsaturated CO), and 1635 (C=C) cm^{-1}.

XV: colorless crystals (81 mg, 64% yield); mp 156–158°; IR (potassium bromide) 3480 (OH), 1740 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

XVI: colorless crystals (115 mg, 90% yield); mp 198–200°; IR (potassium bromide) 3460 (OH), 1730, 1715 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

XVII: colorless crystals (83 mg, 65% yield); mp 145–147°; IR (potassium bromide) 3400 (OH), 1720, 1710 (ester and lactone CO), 1670 (α,β-unsaturated CO), and 1635 (C=C) cm^{-1}.

XVIII: colorless crystals (124 mg, 82% yield); mp 147–149°; IR (potassium bromide) 3460 (OH), 1730, 1710 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1640 (C=C) cm^{-1}.

XIX: colorless crystals (103 mg, 80% yield); mp 253–255°; IR (potassium bromide) 3400 (OH), 1730, 1710 (ester and lactone CO), 1690 (α,β-unsaturated CO), and 1630 (C=C) cm^{-1}.

XXXI: colorless crystals (16.4 mg, 11% yield); mp 168–170°; IR (potassium bromide) 3400 (OH), 1730, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), and 1630 (C=C) cm^{-1}.

General Method for Synthesis of XX–XXX—12-Acetoxybrusatol-

Table III—Analytical Data of Bisbrusatolyl and Brusatolyl Esters and Related Compounds

Compound	Empirical Formula	Theory, % C	Theory, % H	Found, % C	Found, % H	Characteristic Ions[a] in the Field Desorption Mass Spectrum
III	$C_{55}H_{64}O_{24}$ (1108)					520 $[a + H]^+$
IV	$C_{56}H_{66}O_{24}$ (1122)					502 $[a + H - H_2O]^+$
V	$C_{57}H_{68}O_{24}$ (1136)					502 $[a + H - H_2O]^+$
VI	$C_{58}H_{70}O_{24}$ (1150)					502 $[a + H - H_2O]^+$
VII	$C_{62}H_{78}O_{24}$ (1206)					502 $[a + H - H_2O]^+$
VIII	$C_{60}H_{70}O_{26}$ (1206)					544 $[a + H - H_2O]^+$
	$C_{60}H_{70}O_{26}H_2O$	58.92	5.89	58.98	5.89	
IX	$C_{61}H_{72}O_{26}$ (1220)					544 $[a + H - H_2O]^+$
	$C_{61}H_{72}O_{26}\frac{1}{2}H_2O$	59.56	5.94	59.59	5.95	
X	$C_{62}H_{74}O_{26}$ (1234)					544 $[a + H - H_2O]^+$
XI	$C_{55}H_{68}O_{24}$ (1112)					522 $[b + H]^+$
XII	$C_{57}H_{72}O_{24}$ (1140)					522 $[b + H]^+$
XIII	$C_{56}H_{74}O_{24}$ (1130)					506 $[c + H - H_2O]^+$
XIV	$C_{31}H_{38}O_{12}$ (602)					602 M^+
XV	$C_{31}H_{40}O_{12}$ (604)					605 $[M + H]^+$
XVI	$C_{32}H_{40}O_{14}$ (648)					649 $[M + H]^+$
XVII	$C_{33}H_{42}O_{12}$ (630)					631 $[M + H]^+$
XVIII	$C_{36}H_{42}O_{15}$ (714)					714 M^+
	$C_{36}H_{42}O_{15}\frac{1}{2}H_2O$	59.75	5.94	59.53	5.94	
XIX	$C_{35}H_{38}O_{12}$ (650)					650 M^+
XX	$C_{33}H_{40}O_{13}$ (644)					644 M^+
XXI	$C_{33}H_{42}O_{13}$ (646)					646 M^+
XXII	$C_{34}H_{42}O_{15}$ (690)					691 $[M + H]^+$
XXIII	$C_{35}H_{44}O_{13}$ (672)					672 M^+
XXIV	$C_{38}H_{44}O_{16}$ (756)					756 M^+
	$C_{38}H_{44}O_{16}\frac{1}{2}H_2O$	59.60	5.88	59.99	5.38	
XXV	$C_{35}H_{42}O_{14}$ (686)					686 M^+
XXVI	$C_{35}H_{44}O_{14}$ (688)					688 M^+
XXVII	$C_{36}H_{44}O_{16}$ (732)					732 M^+
XXVIII	$C_{37}H_{46}O_{14}$ (714)					714 M^+
XXIX	$C_{39}H_{42}O_{14}$ (734)					734 M^+
	$C_{39}H_{42}O_{14}\frac{1}{2}H_2O$	62.98	5.78	63.00	5.80	
XXX	$C_{32}H_{38}O_{14}$	59.43	5.92	59.13	6.20	
XXXI	$C_{44}H_{44}O_{13}$ (780)					780 M^+
XXXII	$C_{47}H_{62}O_{14}$ (850)					850 M^+

[a] The most intense ion is reported.

3-yl senecioate (XX), 11,12-diacetoxybrusatol-3-yl senecioate (XXV), 12-acetoxybrusatol-3-yl valerate (XXI), 11,12-diacetoxybrusatol-3-yl valerate (XXVI), 12-acetoxy-brusatol-3-yl ethyl succinate (XXII), 11,12-diacetoxybrusatol-3-yl ethyl succinate (XXVII), 12-acetoxybrusatol-3-yl 3,4-dimethyl-2-pentenoate (XXIII), 11,12-diacetoxybrusatol-3-yl 3,4-dimethyl-2-pentenoate (XXVIII), 12-acetoxybrusatol-3-yl 3′,4′,5′-trimethoxybenzoate (XXIV), 11,12-diacetoxybrusatol-3-yl cinnamoate (XXIX), and 11,12-diacetoxybrusatol-3-yl acetate (XXX) were synthesized. Acetylation of XIV (58.8 mg, 0.098 mmole), XV (49 mg, 0.081 mmole), XVI (69 mg, 0.106 mmole), XVII (84 mg, 0.133 mmole), and XVIII (25 mg, 0.0342 mmole) with 2 ml of dry pyridine–acetic anhydride (1:1) at room temperature for 21–24 hr yielded the corresponding 12 mono- and 11,12 diacetates XX, XXV, XXI, XXVI, XXII, XXVII, XXIII, XXVIII, and XXIV, respectively. Similar acetylation of XIX (50 mg, 0.0769 mmole) for 68 hr gave XXIX. Compound XXX was prepared according to a literature method (14). All of these acetates (XX–XXIX) were purified by preparative TLC using 1:1 chloroform–acetone for XX, XXV, XXII, XXVII, XXIII, and XXVIII, and 10:1 chloroform–acetone for XXI, XXVI, XXIV, and XXIX.

XX: colorless crystals (26.7 mg, 42% yield); mp 180–182°; IR (potassium bromide) 3440 (OH), 1740, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1635 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

XXV: colorless crystals (9.1 mg, 14% yield); mp 126–128°; IR (potassium bromide) 1730, 1710 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1635 (C=C), and 1215 ($OCOCH_3$) cm^{-1}.

XXI: colorless crystals (31 mg, 59% yield); mp 116–118°; IR (potassium bromide) 3480 (OH), 1735 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), and 1222 ($OCOCH_3$) cm^{-1}.

XXVI: colorless crystals (15 mg, 27% yield); mp 169–170°; IR (potassium bromide) 1740 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), 1220, and 1135 ($OCOCH_3$) cm^{-1}.

XXII: colorless crystals (52.2 mg, 71% yield); mp 170–172°; IR (potassium bromide) 3440 (OH), 1730, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1635 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

XXVII: colorless crystals (22.85 mg, 29% yield); mp 218–220°; IR (potassium bromide) 1730 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1645 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

XXIII: colorless crystals (46.2 mg, 52% yield); mp 178–180°; IR (potassium bromide) 3440 (OH), 1735, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1635 (C=C), and 1225 ($OCOCH_3$) cm^{-1}.

XXVIII: colorless crystals (14.8 mg, 16% yield); mp 160–162°; IR (potassium bromide) 1750, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

XXIV: colorless crystals (8.1 mg, 31% yield); mp 178–180°; IR (potassium bromide) 3440 (OH), 1730, 1720 (ester and lactone CO), 1680 (α,β-unsaturated CO), 1640 (C=C), and 1210 ($OCOCH_3$) cm^{-1}.

XXIX: colorless crystals (27.1 mg, 48% yield); mp 268–270° (dec.); IR (potassium bromide) 1735, 1720 (ester and lactone CO), 1690 (α,β-unsaturated CO), 1630 (C=C), and 1220 ($OCOCH_3$) cm^{-1}.

General Synthesis of XXXII—Brusatol-3,11,12-triyl 3,4-dimethyl-2-pentenoate (XXXII) was synthesized. To a solution of 3,4-dimethyl-2-pentenoic acid chloride (107 mg, 0.728 mmole) in dry benzene (10 ml) was added II (104 mg, 0.20 mmole). The mixture was refluxed for 24 hr and purified by preparative TLC (chloroform–acetone, 10:1).

XXXII: colorless crystals (159.5 mg, 94% yield); mp 98–101°; IR (potassium bromide) 1720 (ester and lactone CO), 1685 (α,β-unsaturated CO), and 1635 (C=C) cm^{-1}.

Bruceantin-3-yl 3,4-Dimethyl-2-pentenoate (XXXIII)—Compound XXXIII was prepared according to the method of Okano and Lee (15).

22,23-Dihydrobrusatol (XXXIV)—This was prepared according to the method of Sim *et al.* (14).

Synthesis of XXXV—A solution of XXXIV (190 mg) in absolute ethanol (10 ml) was shaken with hydrogen at 65° and atmospheric pressure for 48 hr with 10% palladium-on-charcoal (200 mg). The filtered solution was evaporated to yield colorless crystals of 3,4,22,23-tetrahydrobrusatol (XXXV) after purification by preparative TLC.

XXXV: mp 252–253°; IR (potassium bromide) 3440 (OH), and 1715

(ester and lactone CO) cm^{-1}. PMR ($CDCl_3$) δ 0.99 (d, J = 6 Hz, 9H, CH_3-4 and two CH_3-23), 1.40 (s, CH_3-10), 3.86 (s, $COOCH_3$), and 6.40 (d, J = 13 Hz, H-15).

Anal.—Calc. for $C_{26}H_{36}O_{11}$: *m/z* 524.2255 (M^+). Found: *m/z* 524.2259.

Compound XXXV could also be prepared by hydrogenation of II (504 mg) in 90% ethanol (100 ml) with platinum oxide (50 mg) in water at room temperature and atmospheric pressure. The reaction mixture was filtered by use of a silica gel column. The filtered solution was evaporated to yield colorless XXXV (498 mg).

Synthesis of XXXVI—To a solution of XXXV (66 mg) in methyl alcohol (10 ml) was added a solution of sodium borohydride (2 mg) in water (5 ml). The mixture was stirred at room temperature for 23 hr and then subjected to preparative TLC (chloroform-acetone 1:1) to give 2-hydroxy-2-deoxytetrahydrobrusatol lactol (XXXVI) as colorless crystals (31.6 mg).

XXXVI: mp 121–123°; IR (potassium bromide) 3440 (OH), 1715 (ester CO), 1040 (cyclic *sec*-OH); PMR ($CDCl_3$) δ 0.91 (d, J = 6 Hz, 9H, CH_3-4 and two CH_3-23), 1.50 (s, CH_3-10), 3.81 (s, $COOCH_3$), 4.13 (m, H-12), 4.20 (m, H-11), 4.60 (m, H-2 and H-3) and 5.52 (m, H-15 and H-16).

Anal.—Calc. for $C_{26}H_{40}O_{11}$: *m/z* 528.2571 (M^+). Found: *m/z* 528.2573.

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Antitumor Agents XLVI: *In Vitro* Effects of Esters of Brusatol, Bisbrusatol, and Related Compounds on Nucleic Acid and Protein Synthesis of P-388 Lymphocytic Leukemia Cells

IRIS H. HALL ˣ, Y. F. LIOU, M. OKANO, and K. H. LEE

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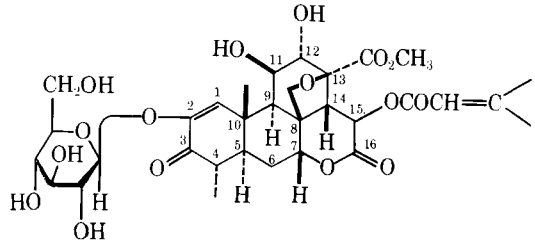
Abstract □ A series of esters of brusatol, bisbrusatol, and bruceantin were shown to have potent antileukemic activity. Antineoplastic activity was correlated with the ability of the compounds to suppress DNA and protein synthesis in P-388 lymphocytic leukemia cells. Compounds with high T/C% values successfully inhibited DNA polymerase activity and purine synthesis. The ability to inhibit protein synthesis during the elongation process also correlated positively with high antileukemic activity in this series of quassinoids. Dihydrofolate reductase activity and basal and adenosine diphosphate stimulated respiration of P-388 cells were also inhibited.

Keyphrases □ Brusatol—esters, *in vitro* effects on nucleic acid and protein synthesis, P-388 lymphocytic leukemia cells □ Bisbrusatol—esters, *in vitro* effects on nucleic acid and protein synthesis, P-388 lymphocytic leukemia cells □ Antitumor agents—*in vitro* effects of brusatol, bisbrusatol, and related compounds on nucleic acid and protein synthesis, P-388 lymphocytic leukemia cells

Bruceantin was first isolated from *Brucea antidysenterica* (1, 2) and is currently in Phase II clinical trials as an antineoplastic agent (3, 4). Brusatol, a derivative of bruceantin, was prepared by Lee *et al.* (5, 6) and shown to be active against P-388 lymphocytic leukemia cell growth (7). This laboratory demonstrated that bruceantin and brusatol reduced nucleic acid and protein synthesis (7, 8), purine synthesis (7), and oxidative phosphorylation processes (8) of P-388 cells. Based on the observation that a number of potent antileukemic agents have an ester in their structure, a series of brusatol related esters as well



I: Bruceoside-A

0022-3549/82/0300-0345$01.00/0