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ANTITUMOR AGENTS, 65.[1] BRUSATOL AND CLEOMISCOSIN-A, ANTILEUKEMIC PRINCIPLES FROM *BRUCEA JAVANICA*

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We reported recently (1) on the isolation and structural elucidation of two novel antileukemic glycosides, bruceoside-A and -B, and brucein-D and -E from the H_2O extract of *Brucea javanica* (L.) Merr. ("Ya-Tan-Tzu"). Further investigation on the $CHCl_3$ extract of the same plant, which showed potent *in vivo* antileukemic activity against P-388 lymphocytic leukemia in mice (2), has now led to the isolation and characterization of brusatol and cleomiscosin-A. Brusatol was previously obtained as the aglycon from an acid hydrolysis of bruceoside-A and -B (1). Brusatol showed potent antileukemic activity in P-388 leukemia (T/C=158 at 0.125 mg/kg/d, ip) (3,4). Cleomiscosin-A is active in the *in vitro* P-388 lymphocytic leukemia (ED_{50}=0.4 μg/ml) and is not active in the *in vitro* KB (ED_{50}>10 μg/ml)[2] system. Cleomiscosin-A was previously isolated from *Cleome viscosa* (6), *Simaba multiflora* (7), and *Soulamea soulameoides* (5).

EXPERIMENTAL

PLANT MATERIAL.—The fruits of *B. javanica* (Simaroubaceae) were procured and identified by H.C. Huang. A voucher specimen is available for inspection at the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

BIOASSAY-DIRECTED ISOLATION AND CHARACTERIZATION OF BRUSATOL AND CLEOMISCOSIN-A.—The active $CHCl_3$ extract (992 g) of *B. javanica* was partitioned to a *n*-hexane soluble portion (228 g) and a MeOH soluble portion (130 g). Repeated column chromatography (silica gel and eluted with $CHCl_3$ and $CHCl_3$ containing increasing proportions of Me_2CO) of the MeOH portion yielded active fractions. From the Me_2CO-$CHCl_3$ (1:1) fraction, 1.4 g of brusatol and 229 mg of cleomiscosin-A were isolated. The identity of both isolates with authentic samples of brusatol and cleomiscosin-A was confirmed by comparative tlc and superimposable ir and nmr spectra. Brusatol: mp 274-277°; ir (KBr) 3515, 3460, 3430, 1730, 1672, and 1630 cm^{-1}; pmr ($CDCl_3$) δ 1.39 (3H, s), 1.85 (3H, d, J=2 Hz), 1.93 (3H, s), 2.19 (3H, s), 2.99 (2H, d, J=16 Hz), 3.15 (1H, d, J=13 Hz), 3.79 (3H, s), 3.80 (1H, d, J=8 Hz), 4.21 (1H, m), 4.26 (1H, m), 4.73 (1H, d, J=8 Hz), 4.80 (1H, m), 5.63 (1H, m), and 6.28 (1H, d, J=13 Hz); ms *m/z* 520 (M^+). Cleomiscosin-A: mp 251-253°; ir (Nujol) 3460 and 1700 cm^{-1}; pmr (C_5D_5N) δ 3.72 (3H, s), 3.81 (3H, s), 3.90 (1H, d, J=12, 5 Hz), 4.33 (1H, d, J=12.5 Hz), 4.50 (1H, d, J=7.5 Hz), 4.96 (1H, s), 5.59 (1H, d, J=7.5 Hz), 6.46 (1H, d, J=10 Hz), 6.73 (1H, s), 7.30 (1H, d, J=8.4 Hz), 7.39 (1H, dd, J=8.4, 2 Hz), 7.42 (1H, d, J=2.5 Hz), 7.43 (1H, d, J=2 Hz), 7.75 (1H, d, J=10 Hz), and 11.37 (1H, s); ms *m/z* 386 (M^+), 328, 180, and 137.

ACETYLATION OF CLEOMISCOSIN-A.—Cleomiscosin-A (127 mg) was treated with Ac_2O-pyridine (1:1, 10 ml) at room temperature. After 15 h, water (15 ml) was added and stirred for 30 min. The solid which separated was collected and recrystallized from $CHCl_3$-MeOH to afford cleomiscosin-A diacetate (91 mg): mp 189-190°; ir (KBr) 3530 and 1720 cm^{-1}; pmr (C_5D_5N) δ 2.00 (3H, s), 2.90 (3H, s), 3.71 (3H, s), 3.85 (3H, s), 4.31 (1H, dd, J=12.5, 4 Hz), 4.55 (1H, dd, J=12.5, 4 Hz), 4.73 (1H, dd, J=7.5, 4 Hz), 5.33 (1H, d, J=7.5 Hz), 6.43 (1H, d, J=10 Hz), 6.76 (1H, s), 7.25 (1H, dd, J=8.4, 2 Hz), 7.31 (1H, d, J=8.4 Hz), 7.41 (1H, d, J=2 Hz), and 7.74 (1H, d, J=10 Hz); ms *m/z* 470 (M^+), 428, 368, 222, and 179.

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[1]For part 64, see Y.F. Liou, I.H. Hall, K.H. Lee, W.L. Williams, Jr., and S.G. Chaney, *Biochim. Biophy. Acta*, **739**, 190 (1983).

[2]Handa, *et al.* reported ED_{50} values for cleomiscosin-A in the *in vitro* P-388 and KB test systems as 2.8 μg/ml and 4.9 μg/ml, respectively (5).

Hiroshi Hikino of the Pharmaceutical Institute, Tohoku University, Japan, for an authentic sample of cleomiscosin-A; Dr. Y.C. Cheng and Mr. Michael Fisher of the Cancer Research Center, and Dr. David L. Harris of the Department of Chemistry, University of North Carolina at Chapel Hill, for biological assay and nmr spectra, respectively.

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