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STUDIES OF THE THYMELAEACEAE II. ANTINEOPLASTIC PRINCIPLES OF *GNIDIA KRAUSSIANA*[1,2]

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ABSTRACT.—The acetone and methanol extracts of *Gnidia kraussiana* roots displayed activity against the P-388 lymphocytic leukemia. Bioactivity-directed fractionation led to the isolation of five daphnane orthoesters, all possessing potent antineoplastic activity. Four of these isolates, gnidilatin (**1**), gnidilatidin (**2**), Excoecaria toxin (**3**), and Pimelea factor P_2 (**4**) have been isolated previously from plants in the Thymelaeaceae and Euphorbiaceae. The fifth compound, kraussianin (**5**), is a new member of the macrocyclic daphnane orthoester series of compounds.

The genus *Gnidia* Linn. is, according to Wagenitz (2), in the subfamily Thymelaeoideae, tribe Thymelaeeae (Gnidieae) in the family Thymelaeaceae. *Gnidia* itself is not clearly delineated. Wagenitz (2) includes species of the genus *Lasiosiphon* as a part of *Gnidia*, while Peterson (3,4) and Gastaldo (5) have merged the genera *Lasiosiphon* and *Arthrosolen* into *Gnidia*. A brief description of the genus is given by Hutchinson (6), while more detailed descriptions are found in Gilg (7), Bentham and Hooker (8), and Pearson (9). *Gnidia* species are distributed primarily in tropical and southern Africa although some species may be found in Madagascar and India (2,6).

Gnidia kraussiana Meisn.[4] is a perennial herb with a swollen, fleshy root, typically with many erect stems. The leaves are usually elliptic (sometimes linear, ovate, or obovate), and the pubescence is variable, glabrous to densely bluish or yellowish, with yellow or orange-yellow flowers. It is distributed throughout tropical, central, and southern Africa, frequently in fire-maintained, upland grasslands such as in Tanzania, western Mozambique, Malawi, northern Nigeria, and Cameroon. In the Sudan, the roots, stem, and leaves are used as a molluscicidal agent (10), and in other parts of Africa, extracts of the roots and leaves are used as an arrow poison, a fish poison, and a homicidal poison (11-14). Medicinally, a decoction of the root has been used for abdominal pain (15), and other preparations have been used for sore throat, as a purgative, and in the treatment of wounds, burns, and snakebites (11-13).[5]

The prior phytochemical work on the genus *Gnidia* is summarized in Table 1 (18-27), and of the greatest significance concerning antileukemic principles is the work by Kupchan and co-workers (25-27), which resulted in the isolation of a number of new diterpene esters of the daphnane type.

Both Me_2CO and MeOH extracts of the roots of *G. kraussiana* were found to be highly cytotoxic (P-388, $ED_{50} \leq 10^{-6}$ μg/ml) (28), and a series of solvent partitions re-

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[1]For Paper I in this series, see reference (1).

[2]This work was presented by R.P.B. in partial fulfilment of the Ph.D. degree requirements of the Graduate College, University of Illinois at the Medical Center, August 1981.

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[4]Synonyms for *G. kraussiana* Meisn. include *Lasiosiphon krausii* (Meisn.), *L. kraussianus* (Meisn.) Burtt Davy, *L. kraussianus* var. *villosus* Burtt Davy, *L. hoepfnerianus* Vatke ex Gilg, *L. affinis* Kotschy & Peyr, *L. kerstingii* Pearson, *L. guineenis* Chev., *Gnidia hoepfnerianus* (Vatke) Gilg, *G. djurica* Gilg and *G. usingensis* Gilg.

[5]In France, extracts of *Lasiosiphon kraussianus* have been patented for use in the treatment of leprosy (16,17). The botanical relationship of this plant material to *G. kraussiana* Meisn. is not known with certainty.

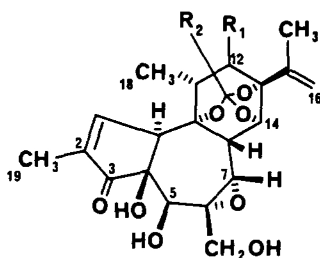
TABLE 1. Isolates of *Gnidia* Species

Compound	Plant	Reference
Coumarins:		
daphnetin-8β-glucoside	*G. polycephala* Gilg.	18
gnidicoumarin	*G. lamprantha* Gilg.	19,20
unidentified	*G. polycephala*	21
Flavonoids:		
kaempferol-3-(*p*-coumarpyl)-O-β-D-glucopyranoside	*G. kraussiana* Meisn.	22
γ-Pyrones:		
maltol	*G. kraussiana*	22
Lignans:		
gnidifolin	*G. latifolia* Gilg.	23
Sesquiterpenes:		
gnididione	*G. latifolia*	24
Diterpenes:		
gnidicin	*G. lamprantha*	25
gnididin	*G. lamprantha*	25
gnidiglaucin	*G. glaucus* Fres.	26
gnidilatidin	*G. latifolia*	26
gnidilatidin-20-palmitate	*G. latifolia*	26
gnidilatin	*G. latifolia*	26
gnidilatin-20-palmitate	*G. latifolia*	26
gnidimacrin	*G. subcordata* (Meisn.) Engl.	27
gnidimacrin-20-palmitate	*G. subcordata*	27
gniditrin	*G. lamprantha*	25
Sterols:		
β-sitosterol	*G. kraussiana*	22
β-sitosterol-β-D-glucoside	*G. kraussiana*	22
Lipids:		
n-eicosanol	*G. kraussiana*	22
1-glyceryl octacosanoate	*G. kraussiana*	22
n-octacpsanol	*G. kraussiana*	22

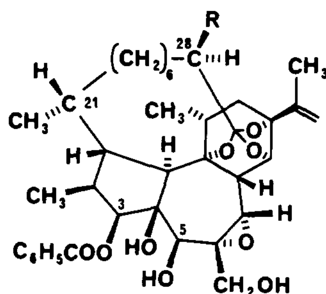
sulted in a number of active fractions. Tlc comparison of the active fractions allowed several to be combined. The most active of these was a light petroleum extract which exhibited $ED_{50} \leq 10^{-9}$ μg/ml. Extensive chromatography on silica gel and Sephadex LH-20, followed by preparative tlc on silica gel, permitted the isolation of two daphnane esters, gnidilatin and gnidilatidin, previously isolated from *Gridia latifolia* (26), Excoecaria toxin (**3**), and Pimela factor P_2 (**4**), and a new daphnane ester, kraussianin, to which we have assigned the structure **5**.

A weak molecular ion at *m/z* 528 was observed for Excoecaria toxin consistent with a molecular formula of $C_{30}H_{40}O_8$, and the base peak at *m/z* 151 is characteristic of the decadienoyl acylium ion ($C_{10}H_{15}O$). Comparison of the pmr spectrum of the isolate with that of montanin (**6**) (29) confirmed that this compound is a daphnane orthoester having a 6,7-α-epoxide. Subtraction of the nucleus from the molecular formula indicated a side chain having the formula C_9H_{15}. Comparison of the chemical shifts and multiplicities of the four olefinic protons with published values for similar protons in huratoxin (30) and yuanhuacine (31) suggests that the stereochemistry and location of the two double bonds in the side chain are the same. The data indicate the structure **3** for the isolate corresponding to that of Excoecaria toxin, a piscicidal constituent from *Excoecaria agallocha* L. (Euphorbiaceae) (32). The observed values are in very close agreement with the reported spectral properties.

Gnidilatidin (**2**) also showed a weak molecular ion, in this instance at *m/z* 648, with intense fragment ions at *m/z* 151 and 105. The molecular ion suggests a molecular for-



		R_1	R_2
1	Gnidilatin	$OCOC_6H_5$	$(CH_2)_8CH_3$
2	Gnidilatidin	$OCOC_6H_5$	$(CH{=}CH)_2(CH_2)_4CH_3$
3	Excoecaria toxin	H	$(CH{=}CH)_2(CH_2)_4CH_3$
6	Montanin	H	$(CH_2)_{10}CH_3$
7	Gnididin	$OCO(CH{=}CH)_2(CH_2)_4CH_3$	C_6H_5



		R
4	Pimelea factor P_2	H
5	Kraussianin	OH

mula $C_{37}H_{44}O_{10}$ and, as in **3**, the ion at *m/z* 151 indicates the presence of a decadienoyl ester unit. The strong fragment ion at *m/z* 105 is characteristic of the benzoyl acylium ion (C_7H_5O). Comparison of the pmr spectrum of the isolate with that of **3** indicated them to be quite similar, except for the five-proton multiplet at δ 7.40-7.90, due to the benzoate moiety, and a one-proton singlet at δ 5.21. This signal is consistent with that of an equatorial (α) methine proton at C-12 in a 12β-acyloxy daphnane derivative such as gnididin (25) or gnidilatidin (26). That the isolate was identical to gnidilatidin (**2**) was established from a primary absorption band at 232 nm, consistent with the presence of an isolated diene system as in **2**, rather than the conjugated dienoate system (260 nm) found in **7**. All of the observed spectral characteristics are in close agreement with the published values for gnidilatidin (**2**) (26).

Gnidilatin (**1**) displayed a molecular ion at *m/z* 652, 4 amu higher than that observed for **2**, and the intense fragments at *m/z* 155 and 105 suggested that four hydrogens have been added to the side chain. In agreement, the pmr spectrum revealed the absence of four olefinic protons and an increase in the intensity of the side-chain methylene signal. The spectral data were found to be in very close agreement with those established for gnidilatin (**1**), which was isolated previously with **2** from *G. latifolia* (26).

A weak molecular ion was observed for Pimelea factor P_2 (**4**) at *m/z* 638 in agreement with a molecular formula $C_{37}H_{50}O_9$, and the intense fragment ion at *m/z* 105 indicated the presence of a benzoate ester. The pmr spectrum now showed three methyl doublets (δ 0.83, 1.06, and 1.44) and a single vinylic methyl singlet at δ 1.73, indicat-

ing that the cyclopentenone unit was now saturated. Furthermore, the signal for H-10 (δ 3.12, d, J=10.5 Hz) indicates that C-1 carries only one proton and that the substituent at C-1 has the α-stereochemistry. Attachment of the benzoate ester at C-3, rather than at C-5 or C-12, was established by the chemical shift and multiplicity (δ 5.07, d) of H-3. The J value for H-3 indicates that the substituents at C-2 and C-3 are both β (27). Comparison of the observed spectral data with those reported for Pimelea factor P_2 (**4**) (33,34) and with an authentic sample (35) established the identity. Pimelea factor P_2 has been isolated from *Pimelea prostrata* Willd. (33,36), *Pimelea lingustrina* Labill. (34), *Pimelea linifolia* Sm. (34), *Dirca occidentalis* A. Gray (35), and *Daphnopsis racemosa* Griseb. (37).

Kraussianin displayed a molecular ion at *m/z* 654, analyzing for $C_{37}H_{50}O_{10}$, one oxygen atom more than **4**, and the intense ion at *m/z* 105 indicated that a benzoate ester was still present. The presence of a 6,7-α-epoxide was indicated by the chemical shift and multiplicity of H-7 (δ 3.32, s), H-8 (δ 3.01, d), and H-10 (δ 2.88, d). The absence of coupling between H-7 and H-8 requires an α-orientation of H-8 for a dihedral angle approximately 90°. The chemical shift of H-10 indicates that it is α-oriented and proximate to the epoxide, and the magnitude of the coupling constant requires a β-oriented H-1. This is consistent with the absence of a 1,2-double bond and the presence of a 1α-alkyl substituent. The location of the benzoate ester and the stereochemistry of the substituents at C-2 and C-3 were established by comparison with the corresponding groups in **4**. The three-proton doublet at δ 0.97 establishes C-18 to be a methyl group.

Comparison of the chemical shift and multiplicity of the C-16 protons in kraussianin (δ 4.86, 5.09, 2 x br s) with the published values for Pimelea factor P_2 (δ 4.95, 4.85, 2 x br s) (33), gnidilatidin (δ 5.01, br s) (26), and gnidimacrin (δ 4.85, 5.09, 2 x br s) (27) demonstrates the absence of oxygenation at C-12 and suggests the presence of a hydroxyl group at C-28 of the macrocyclic chain, as in gnidimacrin.

The *R* configuration at C-28, established in gnidimacrin by X-ray crystallography, is required to place the C-28 hydroxyl group in the region of the protons at C-16. This is the only position in the macrocyclic chain where such a spatial proximity between the hydroxyl group and the protons at C-16 is possible.

The configuration at C-21 in the macrocyclic chain is proposed on the basis of comparison of the chemical shift of the methyl group (C-30) in kraussianin (δ 1.43) with the corresponding group in gnidimacrin (δ 1.42) (36). Dreiding models of a low-energy conformation (side-chain protons staggered) indicated that the methyl group of a compound with 21*R* configuration (as established for gnidimacrin by X-ray crystallography) is substantially less hindered than the corresponding methyl in a 21*S* compound. The resonance for 30-H_3 in a 21*S* derivative would be expected to be upfield of the corresponding signal in a 21*R* compound. Kraussianin is, therefore, assigned the structure **5**.

Samples of the isolates from AB-1 were evaluated in the P-388 lymphocytic leukemia test system *in vitro*. The results are summarized in the Experimental section. All of the samples were highly cytotoxic, with Excoecaria toxin (**3**) and kraussianin (**5**) showing $ED_{50} \leq 10^{-4}$ μg/ml in the P-388 test system. However, it is apparent that the biological activity of the crude fractions (Table 2) is greater than that of the isolates. For this reason, we will continue to study the active fractions with a view to establishing the nature of these constituents. Some variability was observed in repetitive testing of the most highly cytotoxic fractions, and this factor may also account, in part, for the quantitative difference in activity between the crude fraction and the isolates.

Gnidilatin (**1**) and gnidilatidin (**2**) have previously shown *in vivo* antileukemic activity (26). Kraussianin (**5**) displayed T/C 152% at 30 μg/kg in the P-388 test system *in vivo*. Evaluation of the remaining isolates *in vivo* will be conducted when additional

TABLE 2. Biological Activity of the Intial Fractions from the Fractionation of *Gnidia kraussiana* Roots

Fraction	ED_{50}, µg/ml[a]	P-388 Lymphocytic Leukemia Best T/C (%), mg/kg[b]
A	$<10^{-6}$	113/0.25
A-1	$<10^{-10}$	109/0.12
A-2	1.3×10^{-9}	147/0.12
A-3	$<10^{-4}$	119/1.0
A-4	$<10^{-4}$	107/0.5
A-5	0.69	112/0.25
A-6	38.0	105/0.25
B	6×10^{-6}	115/0.5
B-1	1.6×10^{-9}	122/0.12
B-2	4.4×10^{-8}	124/0.25
B-3	0.003	123/2.0
B-4	2.6	90/2.0
B-5	8.0	108/0.5
B-6	77.0	117/0.6

[a]A fraction is considered active in the 9PS5 system if it has an ED_{50} of ≤ 20 µg/ml (28).
[b]A fraction is considered active in the 3PS31 system if it elicits a mean prolongation of life (T/C) of 130% or more (28).

material is available and when further compounds have been obtained from other active fractions.

Discussions relating biological activity with the importance of individual structural units within the molecule are regarded as premature at this time.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were obtained with a Beckman model DB-G spectrophometer, and the ir spectra with a Beckman model IR 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}; absorption bands are recorded in wave numbers (cm^{-1}). Pmr spectra were recorded in $CDCl_3$ with a Varian T-60A instrument operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard, and chemical shifts are recorded in δ (ppm). Mass spectra were obtained with a Varian MAT 112S double focusing spectrometer at 70 eV.

PLANT MATERIAL.—Dried root material of *G. kraussiana* (45 kg) was obtained from Dr. P. Tubery, Laboratorie de Phanerogamie, Museum d'Histoire Naturelle, 15 Rue Buffon, Paris, France. The plant material was collected in Chad during the summer of 1979 and identified by Dr. J. Raynal, Laboratorie de Phanerogamie, Museum d'Histoire Naturelle, Paris, France. A voucher specimen documenting the collection is deposited in the herbarium of that institution.

EXTRACTION OF PLANT MATERIAL.—Coarsely chopped roots of *G. kraussiana* (15 kg) were percolated with distilled Me_2CO (40 liters) at room temperature for 3 days. This process was repeated twice, and the combined Me_2CO extracts were concentrated *in vacuo* at 30° to afford a thick, dark red syrup (approximately 200 g) designated as Fraction A.

After removal of the Me_2CO extract, the plant material was allowed to air dry for 24 h. The marc from the Me_2CO extraction was then extracted in the same manner using distilled MeOH (3×40 liters). The pooled MeOH extracts were concentrated *in vacuo* at 30° to afford a dark, red-brown, semisolid residue (approximately 1200 g) designated as Fraction B.

INITIAL FRACTIONATION OF FRACTION A.—The Me_2CO extract, Fraction A, was suspended in MeOH-H_2O (1:1, 6 liters) and partitioned against light petroleum (6×6 liters). The combined light petroleum fractions were concentrated to a volume of 1 liter and back extracted with MeOH-H_2O (1:1, 1 liter). The aqueous fraction was added to the original hydroalcoholic extract. After drying (Na_2SO_4), the solvent was removed from the light petroleum fraction *in vacuo* at 30° to afford a dark amber syrup (82 g) designated as Fraction A-1.

The hydroalcoholic extract was then extracted with H_2O-saturated Et_2O (5×5 liters). After drying (Na_2SO_4) and removing the solvent *in vacuo* at 30°, the Et_2O soluble fraction was redissolved in H_2O-satu-

rated Et_2O and back extracted with $MeOH$-H_2O (1:1, 1 liter). The aqueous phase was added to the original hydroalcoholic extract. Extraction of the ethereal fraction with 1% aqueous Na_2CO_3, drying (Na_2SO_4), and removal of Et_2O *in vacuo* at 30° yielded a dark orange-brown residue (9.18 g, Fraction A-2).

Partition of the aqueous Na_2CO_3 fraction against H_2O-saturated EtOAc (10×2 liters, Fraction A-3), then H_2O-saturated *n*-BuOH (4×750 ml, Fraction A-4), followed by drying of the organic phases (Na_2SO_4) and removal of solvent *in vacuo* at 30° and 65°, respectively, yielded brown syrups (4.65 g and 10.69 g, respectively).

After adjusting to pH 6 (2 N HCl), the residual aqueous phase was re-extracted with EtOAc (3×500 ml). Drying of the organic phase (Na_2SO_4) and removal of solvent *in vacuo* at 35° yielded a dark red residue (18.40 g, Fraction A-7).

The resulting aqueous fraction was then extracted with *n*-BuOH (6×500 ml). Removal of *n*-BuOH *in vacuo* at 60° yielded a dark brown, solid residue (12.19 g, Fraction A-8). The residual aqueous fraction was freed of organic solvent, then lyophilized to yield a solid residue (23.40 g, Fraction A-9).

INITIAL FRACTIONATION OF FRACTION B.—The MeOH extract, Fraction B, was fractionated by solvent partition in a manner analogous to that used for fractionation of the Me_2CO extract, Fraction A, to afford Fractions B-1 through B-9.

BIOLOGICAL ACTIVITY OF THE INITIAL FRACTIONS.—Samples of Fractions A through A-9 and B through B-9 were evaluated in the P-388 lymphocytic leukemia test system *in vivo* and *in vitro* by the National Cancer Institute according to established protocols (28). The results of these evaluations are presented in Table 2. On the basis of similarity of biological activity and qualitative similarity by tlc, it was decided to combine Fraction A-1 with B-1 to form AB-1, A-2 with B-2 to form AB-2, A-3 with B-3 to form AB-3, and A-7 with B-7 to form AB-7.

FRACTIONATION OF AB-1.—A sample of AB-1 (100 g) was dissolved in light petroleum (1.6 liters) and extracted with $MeOH$-H_2O (5:1, 4×1.6 liters). The solvent was removed *in vacuo* at 30° and residual H_2O removed azeotropically with C_6H_6 to yield a dark-brown, semisolid residue (26.6 g, Fraction AB-1-1).

COLUMN CHROMATOGRAPHY OR FRACTION AB-1-1.—Fraction AB-1-1 (26.6 g) was chromatographed over a column of silica gel-60[6] (1.5 kg, 7.5×75 cm) slurry-packed in $CHCl_3$. Elution started with $CHCl_3$, followed by mixtures of $CHCl_3$ and MeOH of increasing polarity, and finally MeOH. Fifty-six fractions of 2 liters each were collected. Samples of representative fractions were evaluated in the P-388 test system *in vitro*, to afford the following active fractions (fraction number, ED_{50} in μg/ml): 11, 2.9; 14, 1.2×10^{-5}; 15, 5.5×10^{-6}; 16, 1.0×10^{-6}; 18, 1.4×10^{-5}; 24, 7.3×10^{-3}; 27, 0.34; 28, 6.8×10^{-2}; 33, 0.77; 35, 8.5×10^{-3}; 40, 0.22; 47, 0.12; and 52, 3.3×10^{-2}. Chromatography has been conducted on the most active of these fractions.

Chromatographic Separation of Fraction 15.—Fraction 15 from the previous column was chromatographed over a column of silica gel-60 (250 g, 2.5×97 cm) slurry-packed in $CHCl_3$. Elution with $CHCl_3$-MeOH (49:1) afforded 120 fractions (20 ml each), which were compared by tlc and grouped into nine subfractions.

Subfraction 3 from above was chromatographed over a column of Sephadex LH-20[7] (5×95 cm) packed in MeOH. Elution with MeOH afforded 140 fractions (10 ml each) that were grouped into six main fractions on the basis of similarity by tlc.

Preparative tlc of fraction 4 from the Sephadex column on silica gel-60 F_{254}[6] eluting with light petroleum-Me_2CO (2:1) and developing twice, followed by visualization under uv light, permitted the delineation of two main bands at Rf 0.30 and 0.27. Further preparative tlc of the band at Rf 0.30 eluting with the same solvent system afforded a pale yellow oil (13.8 mg, 9.2×10^{-7}%) identified as gnidilatin (**1**) and having the following physical and spectral properties: $[\alpha]^{24}D$ +39.6° (MeOH); ir, ν max (KBr) 3450, 2940, 2860, 2730, 1698 and 710 cm^{-1}; uv, λ max (MeOH) 231 (log ϵ 4.23), 266 (3.01), and 280 nm (2.78); pmr, (60 MHz, $CDCl_3$) δ 1.35 (3H, d, J=7.2 Hz, 18-H_3), 1.78 (3H, s, 19-H_3), 1.84 (3H, s, 17-H_3), 2.52 (1H, q, J=7.2 Hz, 11-H), 3.60 (1H, d, J=2.5 Hz, 8-H), 3.60 (1H, s, 7-H), 3.86 (2H, br s, 20-H_2), 3.86 (1H, s, 10-H), 4.21 (1H, s, 5-H), 4.83 (1H, d, J=2.5 Hz, 14-H), 4.98 (2H, br s, 16-H_2), 5.18 (1H, s, 12-H), 7.50 (1H, br s, 1-H), and 7.40-7.90 (5H, m, 5×Ar-H); ms, *m/z* (rel. int.) 652 (M^+, 2%), 155 (5), and 105 (100). Comparison of the physical data of the isolate with those reported previously (26) established identity with gnidilatin (**1**).

Further preparative tlc of the band at Rf 0.27 in the same solvent system afforded a pale yellow oil (67.1 mg, 4.5×10^{-6}%) identified as gnidilatidin (**2**) and having the following physical and spectral prop-

[6]E. Merck, Darmstadt, West Germany.

[7]Pharmacia Fine Chemicals, Inc., Piscataway, NJ.

erties: $[\alpha]^{24}D$ +57.1° (MeOH); ir, ν max (KBr) 3450, 2930, 1710, 1695, 1628, 1250, 970, and 710 cm^{-1}; uv, λ max (MeOH) 232 (log ϵ 4.76), 266 (3.16), and 280 nm (2.94); pmr, (60 MHz, $CDCl_3$) δ 1.36 (3H, d, J=7.2 Hz, 18-H_3), 1.75 (3H, br s, 19-H_3), 1.86 (3H, s, 17-H_3), 2.55 (1H, q, J=7.2 Hz, 11-H), 3.60 (1H, s, 7-H), 3.60 (1H, d, J=2.2 Hz, 8-H), 3.83 (2H, br s, 20-H_2), 3.83 (1H, s, 10-H), 4.16 (1H, s, 5-H), 4.90 (1H, d, J=2.2 Hz, 14-H), 5.01 (2H, br s, 16-H_2), 5.21 (1H, s, 12-H), 5.53-6.76 (4H, m, 4×olefinic-H), 7.50 (1H, br s, 1-H), and 7.40-7.90 (5H, m, 5×Ar-H); ms, m/z (rel. int.) 648 (M^+, 3%). 151 (44), and 105 (100). Comparison of the physical data of the isolate with those reported previously (26) established identity with gnidilatidin (**2**).

Chromatographic Separation of Fractions 16-22.—Fractions 16-22 from the chromatography of Fraction AB-1-1 were combined and rechromatographed over a column of silica gel-60 (500 g, 4×90 cm) slurry-packed in $CHCl_3$. Elution was commenced with $CHCl_3$ followed by mixtures of $CHCl_3$ and MeOH of increasing polarity and finally MeOH. Sixty-eight fractions (250 ml each) were collected and grouped into 19 fractions based on their similarity by tlc.

Subfractions 11 and 12 from above were combined and chromatographed over a column of Sephadex LH-20 (1.5×40 cm) packed in MeOH. Elution with MeOH afforded 127 fractions (5 ml each), which were combined to form subfractions after tlc comparison.

Preparative tlc of fraction 4 from the Sephadex column on silica gel-60 F_{254} eluting with $CHCl_3$-MeOH (19:1) with visualization under uv light permitted delineation of a major component (Rf 0.51) that was rechromatographed in the same solvent system to yield a pale yellow oil (76 mg, 5.0×10^{-6}%) identified as Excoecaria toxin (**3**) and having the following physical and spectral properties: $[\alpha]^{24}D$ +52.8° (MeOH); ir, ν max (KBr) 3400, 2930, 1700, 1685, 1295, and 1000 cm^{-1}; uv, λ max (MeOH) 232 (log ϵ 4.40), and 280 nm (2.71); pmr, (60 MHz, $CDCl_3$) δ 1.19 (3H, d, J=8 Hz, 18-H_3), 1.78 (6H, br s, 17-H_3 and 19-H_3), 2.91 (1H, d, J=2.5 Hz, 8-H), 3.43 (1H, br s, 7-H), 3.82 (2H, br s, 20-H_2), 4.25 (1H, s, 5-H), 4.43 (1H, d, J=2.5 Hz, 14-H), 4.90 (1H, br s, 16-H), 5.01 (1H, br s, 16-H), 5.55-6.90 (4H, m, 4×olefinic-H), and 7.59 (1H, br s, 1-H); ms, m/z (rel. int.) 528 (M^+, 6%) and 151 (100). Comparison of the physical data of the isolate with those of Excoecaria toxin (**3**) established the identity (32).

Preparative tlc of fraction 6 from the Sephadex column on silica gel-60 F_{254} eluting with $CHCl_3$-MeOH (97:3) and developing twice, followed by visualization under uv light, permitted the delineation of a major band (Rf 0.25). Further preparative tlc of this material in the same solvent system afforded a pale yellow oil (7.3 mg, 4.9×10^{-7}%) identified as Pimelea factor P_2 (**4**) having the following physical and spectral properties: $[\alpha]^{24}D$ +23.2° (MeOH); ir, ν max (KBr) 3400, 1700, 1640, and 650 cm^{-1}; uv, λ max (MeOH) 230 (log ϵ 4.05), 266 (2.84), and 280 nm (2.66); pmr, (60 MHz, $CDCl_3$) δ 0.83 (3H, d, J=5.9 Hz, 18-H_3), 1.06 (3H, d, J=6.7 Hz, 19-H_3), 1.44 (3H, d, J=6.9 Hz, 30-H_3), 1.73 (3H, s, 17-H_3), 2.87 (1H, d, J=2.4 Hz, 8-H), 3.12 (1H, d, J=10.5 Hz, 10-H), 3.34 (1H, s, 7-H), 3.83 (2H, br s, 20-H_2), 4.11 (1H, br s, 5-H), 4.27 (1H, d, J=2.4 Hz, 14-H), 4.85 (1H, s, 16-H), 4.95 (1H, s, 16-H), 5.07 (1H, d, J=5 Hz, 3-H), and 7.45-8.12 (5H, m, 5×Ar-H); ms, m/z (rel. int.) 638 (M^+, 2%) and 105 (100). Comparison of the physical data of the isolate with those reported previously (33,34) and direct comparison with an authentic sample (35) established the identity with Pimelea factor P_2 (4).

Subfraction 16 from the rechromatography of Fractions 16-22 was itself rechromatographed over a column of Sephadex LH-20 (5×95 cm) packed in MeOH. Elution with MeOH afforded 160 fractions (10 ml each), which were combined to form nine subfractions after tlc comparison.

Preparative tlc of fraction 7 from the Sephadex column on silica gel-60 F_{254} eluting with $CHCl_3$-MeOH (19:1) and developing twice, followed by visualization under uv light, permitted the delineation of a major component (Rf 0.31). Further preparative tlc on silica gel-60 F_{254} eluting with light petroleum-Me_2CO (3:2) and developing twice afforded a colorless oil (138 mg, 9.2×10^{-6}%) identified as kraussianin (**5**) and having the following physical and spectral properties: $[\alpha]^{24}D$ +14.9° (MeOH); ir, ν max (KBr) 3525, 1730, 1605, and 710 cm^{-1}; uv, λ max (MeOH) 230 (log ϵ 4.08), 266 (2.89), and 280 nm (2.79); pmr, (60 MHz, $CDCl_3$) δ 0.97 (3H, d, J=6.4 Hz, 18-H_3), 1.19 (3H, d, J=8.0 Hz, 19-H_3), 1.43 (3H, d, J=8.6 Hz, 30-H_3), 1.76 (3H, s, 17-H_3), 2.88 (1H, d, J=11.2 Hz, 10-H), 3.01 (1H, d, J=1.8 Hz, 8-H), 3.32 (1H, s, 7-H), 3.80 (2H, br s, 20-H_2), 4.03 (1H, s, 5-H), 4.31 (1H. d, J=1.8 Hz, 14-H), 4.86 (1H, br s, 16-H), 4.97 (1H, d, J=4.4 Hz, 3-H), 5.09 (1H, br s, 16-H), and 7.40-8.15 (5H, m, 5×Ar-H); ms, m/z (rel. int.) 654 (M^+, 0.5) and 105 (100). Mass measurement, Obsd. 654.3400, calcd for $C_{37}H_{50}O_{10}$ 654.3402.

BIOLOGICAL ACTIVITY OF THE ISOLATES.—Each of the isolates was found to be active in the P-388 lymphocytic leukemia system in cell culture (28): gnidilatin (**1**), NSC-266489, $ED_{50}<10^{-3}$ µg/ml; gnidilatidin (**2**), NSC-261422, $ED_{50}<10^{-3}$ µg/ml; Excoecaria toxin (**3**), NSC-363005, ED_{50} 0.00024 µg/ml; Pimelea factor P_2 (**4**), NSC-334694, ED_{50} 0.0023 µg/ml; kraussianin (**5**), NSC-363005, ED_{50} 0.00078 µg/ml.

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ERRATUM

Tian-Shung Wu, Hiroshi Furukawa, and Chang-Sheng Kuoh: Triterpenoids from *Humata pectinata*, *J. Nat. Prod.*, **45**, 721 (1982).

The authors make the following correction:

In this paper, isolations of hopa-3β-ol (**1**) and hopan-3β-yl acetate (**2**) from *Humata pectinata* were reported. However, in the results of gas chromatographic analysis, the samples assigned to **1** (mp 236-238°) and **2** (mp 324-326°) were shown to be a mixture of tetrahymanol and neriifoliol (1:3), and tetrahymanyl acetate, respectively.

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