Reduction of 3,4-Diacetoxydibenz[*a*,*h*]anthracene (5l). Reaction of **5l**[36] (79 mg, 0.2 mmol) with $NaBH_4$ (200 mg) in ethanol (15 mL) under air for 70 h yielded after recrystallization from THF *trans*-dihydro diol **4l**, 44 mg (70%).

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Registry No. **1a**, 524-42-5; **1b**, 655-04-9; **1c**, 573-12-6; **1d**, 4733-11-3; **1e**, 82120-27-2; **1f**, 74877-25-1; **1g**, 82120-26-1; **1h**, 82120-28-3; **1i**, 65199-11-3; **1j**, 82120-25-0; **1k**, 82120-24-9; **1l**, 70644-35-8; **3a**, 574-00-5; **4a**, 771-16-4; **4b**, 4841-37-6; **4c**, 60917-41-1; *cis*-**4d**, 61616-82-8; *trans*-**4d**, 569-20-0; **4f**, 60967-89-7; **4g**, 34501-24-1; *cis*-**4h**, 82120-22-7; *trans*-**4h**, 68151-04-2; **4i**, 57404-88-3; *cis*-**4j**, 56484-47-0; *trans*-**4j**, 58886-98-9; **4k**, 66788-06-5; **4l**, 66267-19-4; **5a**, 6336-79-4; **5b**, 74877-26-2; **5e**, 84040-73-3; **5h**, 84040-74-4; **5k**, 84040-75-5; **5l**, 70644-34-7.

(36) We thank H. Frank, University of Mainz, for a sample of 3,4-diacetoxydibenz[*a*,*h*]anthracene.

Microminutin, a Novel Cytotoxic Coumarin from *Micromelum minutum* (Rutaceae)

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Introduction

Previous studies of *Micromelum* species, family Rutaceae,[1-4] have revealed a number of unique coumarins, e.g., micromelin (**1**, Chart I) in which an α,β-unsaturated γ-lactone is bound to a coumarin nucleus. Cassady et al.[3] have recently reported that **1** displays in vivo activity in the P-388 lymphocytic leukemia test system.[5]

The present study is concerned with the isolation and structure elucidation of microminutin (**2**), a novel cytotoxic coumarin from the leaves of *M. minutum* (Forst. f.) Seem. (syn. *Micromelum pubescens* Blume), collected in Thailand. The pyranoquinoline alkaloid flindersine **3** was also isolated in the course of these studies. Microminutin (**2**) was isolated in almost 1% yield from the plant, and its structure was determined from the following spectroscopic studies.

Discussion

The molecular formula $C_{15}H_{12}O_6$ indicated for the isolate was established by high-resolution mass spectrometry. The infrared spectrum showed a very strong, wide carbonyl band at 1740 cm^{-1} and suggested the presence of two unsaturated lactones or of one unsaturated lactone and one saturated ester group, an ambiguity that was resolved by NMR analysis. The IR spectrum did, however, indicate

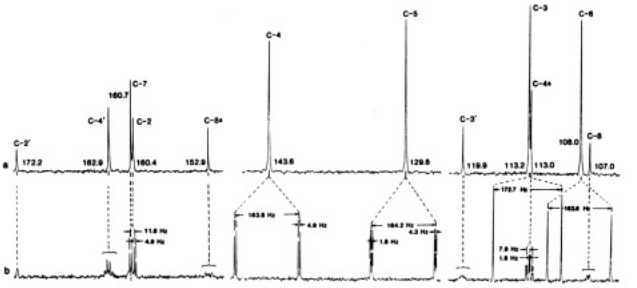

Figure 1. Carbon-13 NMR spectrum (25.05 MHz) of microminutin (**2**): (a) proton noise decoupled (PND); (b) coupled spectrum.

an absence of hydroxyl groups.

From previous studies[1-4] a coumarin nucleus was suspected for microminutin. This and the location and nature of the substituents on the nucleus were deduced by a combination of ^1H and ^{13}C NMR spectroscopy.

The proton noise decoupled (PND) ^{13}C spectrum of microminutin as well as the coupled ^{13}C spectrum (Figure 1) exhibit resonances of δ 160.4 as a doublet of doublets (J = 4.6, 11.4 Hz), δ 113.2 as a doublet (J = 172.6 Hz), and δ 143.6 as a doublet of doublets (J = 4.9, 163.0 Hz). These three resonance patterns are characteristic[6] of C-2, C-3, and C-4, respectively, of the coumarin nucleus.

The aromatic region of the ^1H NMR spectrum of microminutin at 360 MHz contains only four resonance patterns, all of which are doublets. A pair of doublets (9.5 Hz) at δ 6.25 and 7.68 are characteristic of H-3 and H-4 in a coumarin nucleus.[7] Irradiation of the H-4 doublet at δ 7.68 produces a positive nuclear Overhauser effect (NOE) in the doublet (J = 8.8 Hz) at δ 7.50, which can therefore be assigned to H-5, leaving the doublet at δ 6.93 as H-6. The absence of any other ^1H signals in the aromatic region clearly indicates that positions C-7 and C-8 are substituted and furthermore that their substituents do not possess any aromatic or olefinic protons.

The three-proton singlet at δ 3.90 may be assigned to either aromatic methoxy (OMe) or carbomethoxy (COOMe) protons, and this ambiguity could also be resolved by performing a double resonance experiment. Irradiation at the frequency of this signal shows a strong, positive (23.4%) NOE in the H-6 doublet at δ 6.93. This experiment suggests that the resonance at δ 3.90 should be the protons of a methoxy group located at C-7. Similar irradiation of a resonance from the protons of either a COOMe group at C-7 or a methoxy group at C-8 would not be expected to produce the above effect.

With the coumarin nucleus and its substitution pattern firmly established, attention was turned to the molecular array attached at C-8 in microminutin. From the high-resolution mass spectrum this fragment should have the molecular formula $C_5H_5O_2$ and the nature of this moiety was deduced through total assignment of the ^{13}C and ^1H NMR spectra. As an initial step, the carbon resonances representing the coumarin nucleus and the C-7 methoxy group were assigned by comparison of the ^{13}C spectra of

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Chart I

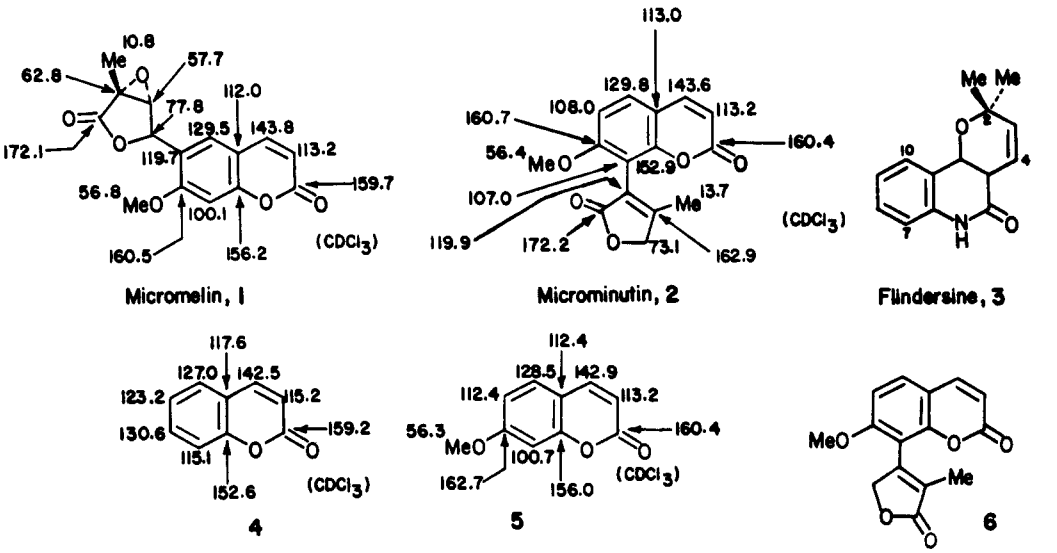

Table I. ^{13}C-H Coupling Constants for Microminutin (2)

C	multiplicity[a]	coupling const, Hz	coupling assignment
2	dd	11.6	H_4
		4.9	H_3
3	d	172.7	H_3
4	dd	163.8	H_4
		4.9	H_5
4a	tt	7.9	H_6, H_3
		1.8	H_5, H_4
5	ddd	164.2	H_5
		4.3	H_4
		1.8	H_6
6	d	163.6	H_6
7		m	OCH_3
8		m	
8a		m	
2′		m	
3′		m	
4′	m	6.7	CH_3[b]
		4.3	CH_2[b]
5′	tq	151.8	H_3′
		5.1	CH_3

[a] dd = doublet of doublets, d = doublet, ddd = doublet of doublets of doublets, tt = triplet of triplets, t = triplet, q = quartet, m = unresolved multiplet. [b] Obtained from low-power single frequency selective decoupling (LPSFSD) experiments.

microminutin with those of coumarin (4), 7-methoxycoumarin (5), and micromelin (1).[3,8,9] Thus the resonances at δ 107.0, 108.0, 113.0, 113.2, 129.8, 143.6, 152.9, 160.4, and 160.7 represent C-8, C-6, C-4a, C-3, C-5, C-4, C-8a, C-2, and C-7, respectively (Figure 1 and Table I). The five remaining ^{13}C resonances in the microminutin spectrum must correspond to the carbon atoms occurring in the C-8 substituent. A correlation of these resonances in the PND and the single-frequency-off-resonance-decoupled (SFORD) spectra provide the following structural assignments: δ 172.2 singlet in PND, remains singlet in SFORD (–OC(O)–); δ 119.2 and 160.9 singlets in PND, remain singlets in SFORD (>C=CHC(O)–); δ 73.1 singlet in PND, becomes triplet in SFORD ($-CH_2OC(O)-$); δ 13.7 singlet in PND, becomes quartet in SFORD ($-C(CH_3)=C<$). Construction of the above structural fragments into a ring framework suggests a γ-butenolide moiety as the C-8

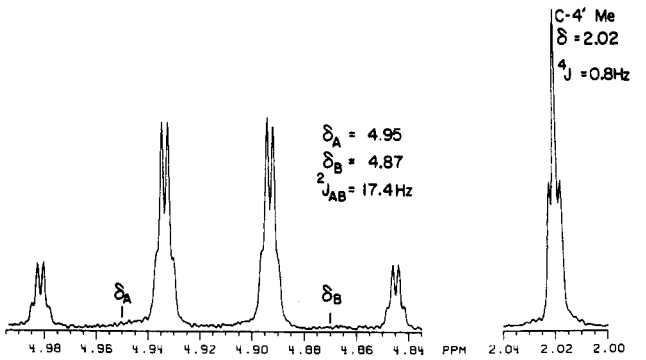

Figure 2. Methylene and olefinic methyl regions in the proton NMR spectrum (360 MHz) of microminutin (2).

substituent on the coumarin nucleus. Additional structural information could be deduced from a consideration of the proton NMR spectral data. The broadened 2 H signal (100 or 200 MHz) centered at δ 4.88 is characteristic of the protons of a methylene group flanked by a lactone oxygen and an olefinic carbon atom, and the slightly broadened 3 H singlet (100 or 200 MHz) at δ 2.02 is typical of a vinyl methyl. At 360 MHz, however, the methylene protons (δ 4.87 and 4.95) were each observed as quartets of doublets due to molecular dissymetry showing ^2J = 17.4 and ^4J = 0.8 Hz (Figure 2). The only remaining ambiguity in the structure of microminutin is therefore the relative location of the vinyl methyl and the carbonyl function in the γ-butenolide moiety. Two alternative structures, **2** and **6**, were therefore considered for microminutin.[10]

Distinction between structures **2** and **6** was achieved independently by (i) application of intramolecular nuclear Overhauser difference spectroscopy and (ii) single frequency selective decoupling of the ^{13}C NMR spectrum of microminutin. Thus, irradiation of the methyl at δ 2.02 (360 MHz) using the NOE difference technique[11-22] leads

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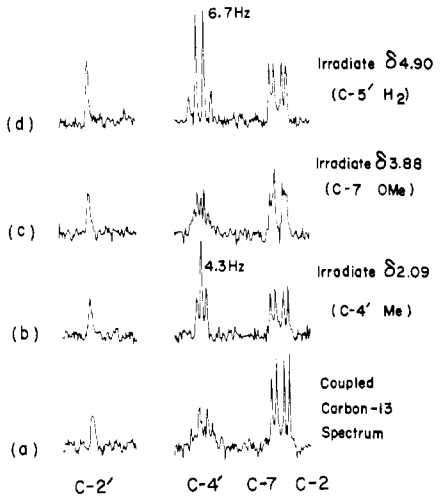

Figure 3. Low-power single frequency selective decoupling $^{13}C\{^1H\}$ spectra of microminutin (2), observation of low-field spectral region.

to a 2.5% NOE for the pattern due to the methylene protons. No appreciable NOE was observed when the methoxyl protons (δ 3.90) were similarly irradiated. The NOE observed for the methylene protons is much smaller than that observed for the aromatic CH because the dominant relaxation of each methylene proton is through its geminal neighbor. Nevertheless there are some contributions from the neighboring C–CH_3 protons that would only occur in structure **2**.

Low-power single frequency selective decoupling (LPSFSD) of the ^{13}C spectrum (25.05 MHz, 50 °C) gave complimentary results (Figure 3). Thus irradiation of the C–CH_3 protons caused the multiplet at δ 162.9 observed in the coupled ^{13}C spectrum to collapse to a triplet ($^3J_{CH}$ = 4.3 Hz). Conversely, irradiation of the methylene proton signal centered at δ 4.90 (100 MHz, ^1H) caused the signal at δ 162.9 to collapse to a quartet ($^3J_{CH}$ = 6.7 Hz). The observation that only *one* carbon signal is affected in these LPSFSD experiments argues in favor of both the methyl and the methylene groups being attached to the *same* sp^2 carbon atom; i.e., microminutin has the structure **2**.

Microminutin (**2**) is the first member of a new series of prenylated coumarins in which neither the "head" nor "tail" of the isoprene unit is attached either to a heteroatom or the coumarin nucleus, but rather an adjacent carbon forms the crucial bond.

Microminutin (NSC-324638) was found to be inactive in the KB cytotoxicity test but did show weak activity (ED_{50} 3.7 μg/mL) in the P-388 lymphocytic leukemia test system in vitro.[5]

Flindersine (**3**) was also obtained from the leaf extract and identified by direct comparison of the spectroscopic data with those of flindersine from *Geijera parviflora*[23] and *Haplophyllum perforatum*.[24] This alkaloid has also been isolated from *Atlantia roxburghiana*,[25] *Flindersia australis*,[26,27] *Haplophyllum tuberculatum*,[28] and *Zanthoxylum coco*.[29,30]

Experimental Section

Plant Material. The leaf material of *Micromelum minutum* (Forst. f). Seem. was collected at San Lam Waterfall, Saraburi Province, Thailand. It was identified by the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. A herbarium specimen is deposited in the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation of Microminutin. Dried powdered leaves (650 g) of *Micromelum minutin* were macerated with 95% EtOH (2 L) for 3 days. After evaporation of the eluent in vacuo (to 200 mL), distilled H_2O (300 mL) and saturated $Pb(OAc)_2$ solution (120 mL) were added, the mixture was centrifuged, filtered, extracted with $CHCl_3$ (3 L), and the $CHCl_3$ fraction was dried (Na_2SO_4). The residue after evaporation (8.5 g) was chromatographed on Si gel eluting with $CHCl_3$ and the main constituent crystallized from absolute EtOH to afford cream-colored rosettes of microminutin (**2**, 6.35 g, 0.98%): mp 154–155 °C; IR (KBr) υ_{max} 1740 (vs), 1675 (m), 1600 (s), 1560 (m), 1495 (m), 1440 (m), 1395 (m), 1285 (s), 1247 (s), 1142 (s), 1108 (s), 1090 (s), 1075 (m), 1060 (m), 1033 (s), 1003 (m), 928 (w), 890 (m), 830 (s), 760 (m), 720 (w), 640 (w), 610 (w), 460 (w), 390 (w) cm^{-1}; UV (EtOH) λ_{max} (log ε) 268 nm (3.59), 321 (4.23); ^1H NMR (360 MHz, $CDCl_3$) δ 2.02 (t, J = 0.8 Hz, 3 H, C_4–CH_3), 3.88 (s, 3 H, C_7–OCH_3), 4.87 (qd, J = 0.8, 17.4 Hz, 1 H, C_5–H), 4.95 (qd, J = 0.8, 17.4 Hz, 1 H, C_5–H), 6.25 (d, J = 9.5 Hz, 1 H, C_3–H), 6.93 (d, J = 8.8 Hz, 1 H, C_6–H), 7.50 (d, J = 8.8 Hz, 1 H, C_5–H), 7.68 (d, J = 9.5 Hz, 1 H, C_4–H); ^{13}C NMR (25.05 MHz, $CDCl_3$), see Figure 1; MS, m/e 272 (M^+, 7), 257 (57), 243 (12), 227 (42), 216 (15), 215 (100), 213 (27), 199 (25), 187 (16), 185 (14), 172 (16), 171 (16), 159 (16), 128 (22). Mass measurement: obsd, 272.0599; calcd for $C_{15}H_{12}O_5$, 272.0685.

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Registry No. Microminutin, 84041-46-3; flindersine, 523-64-8.

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Iodine-Induced Formation of Bicyclo[3.3.0]octane Derivatives from 1,5-Cyclooctadiene

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It has been known that the addition of various pseudohalogens IX (X = NCO, N_3, NO_3) to *cis,cis*-1,5-cyclooctadiene (COD) yields only 1,2-monocyclic adducts and does not give any bicyclic products via transannular π

