The Isolation and Structure of Toxic Principles, Milliamines A, B, and C, from *Euphorbia Millii*

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Toxic compounds were isolated from *Euphorbia Millii* Ch. des Moulins (*Euphorbiaceae*). The structure of the milliamines was established from chemical and spectral evidence. Furthermore, the acute toxicity of milliamine A was examined.

Although *Euphorbia Millii* Ch. des Moulins (Japanese name, Hanakirin) is widely cultivated for decorative purposes, its toxicity is also known. The toxic compound of this plant is of interest. A detailed survey of methanol extracts gave three alkaloidal toxic compounds, namely, milliamines A (**1**),[1] B (**2**),[1] C (**3**).[2] Particularly milliamine A hydrochloride possesses high toxicity (LD_{50}=0.64 mg/kg).

Isolation of Milliamines. Dried roots were extracted with methanol over a period of a few days. The evaporation of methanol under reduced pressure gave a moist oily material, which was extracted with ether several times. The organic layers were concentrated and the residual oily material was subjected to chromatography on silicic acid. The toxic material responded positively to the Dragendorff test. A fraction (responding positively to the Dragendorff test) afforded milliamine A, which was purified as a hydrochloride. Milliamine A hydrochloride was recrystallized from acetone. Milliamines B and C were purified by preparative thin-layer chromatography. Milliamine B hydrochloride was obtained as a powder. Milliamine C was unstable in acidic media, and was purified by repetitive preparative thin-layer chromatography. The physical and spectral data of these new compounds are summarized in Table 1.

The Structure of Milliamine A. Milliamine A (**1**) possesses the molecular formula, $C_{45}H_{49}N_3O_{10}$. Strong hydrogen bonding was present in **1** judging from the absorption in the region of 2800—3600 cm^{-1} in the IR spectrum. Also, the IR spectrum showed the presence of carbonyl groups (1725, 1690, 1670, and 1645 cm^{-1}) including an ester group, which is cleavable by methanolysis. In practice, treatment of milliamine A (**1**) with sodium methoxide afforded an alkaloidal part, compound **4**, and an alcohol (**5**).

Compounds **4** was purified by recrystallization from methanol and possesses the molecular formula $C_{24}H_{23}N_3O_5$: mp 160—161 °C; IR (KBr) 3400, 3250, 2400—3000, 1690, 1670, 1630, 1590, 1510, and 1300 cm^{-1}; NMR (60 MHz, acetone-d_6) δ 2.73 [6H, s, $-N(CH_3)_2$], 3.77 (3H, s, $-COOCH_3$), 6.8—7.6 (8H, m), 7.7—8.1 (2H, m), 8.60 (1H, d of d, J=1.0 and 10 Hz), 9.26, 11.3, and 12.9 (1H each, br s, exchangeable with D_2O). The IR spectrum of compound **4** indicates the presence of α,β-unsaturated ester group (1690 cm^{-1}), and reduction with lithium aluminum hydride of **4** afforded an alcohol (**6**). Furthermore, the presence of two amide

TABLE 1. PHYSICAL CONSTNATS AND SPECTRAL DATA OF MILLIAMINES

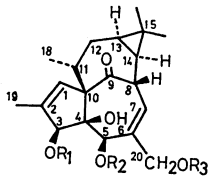
	Milliamine A (**1**) $C_{45}H_{49}N_3O_{10}$	Milliamine B (**2**) $C_{43}H_{47}N_3O_9$	Milliamine C (**3**) $C_{43}H_{47}N_3O_9$
Mp	167—170 °C (hydrochloride)	140 °C (dec) (hydrochloride)	amorphous powder
$[\alpha]_D^{25}$	+6 (*c* 1.4, $CHCl_3$) (hydrochloride)	−14 (*c* 1.4, $CHCl_3$) (hydrochloride)	+11 (*c* 1.0, $CHCl_3$)
UV (MeOH)	232 (ε 37300), 260 (21900) 315 (14100), and 342 nm (4800) (hydrochloride)	227 (ε 43800), 260 (18800), 315 (14600), and 342 nm (5000) (hydrochloride)	227 (ε 43500), 262 (18800), 315 (14500), and 342 nm (4900)
IR	2800—3600, 1725, 1690, 1670, 1645, 1610, 1585, 1530, and 1310 cm^{-1} (KBr, hydrochloride)	2800—3600, 1710, 1685, 1670, 1650, 1610, 1580, 1520, and 1300 cm^{-1} (KBr, hydrochloride)	2800—3600, 1715, 1695, 1680, 1630, 1610, 1580, 1520, and 1300 cm^{-1} ($CHCl_3$)
NMR (60 MHz, $CDCl_3$)	δ 0.5—1.4 (11H, complex pattern), 1.83 (3H, d, J=1.0 Hz, H-19), 2.05 (3H, s), 2.83 (6H, s), 3.64 (1H, s, OH), 3.95 (1H, m, H-5), 4.15 (1H, m, H-8), 4.10 (1H, br s, OH), 4.43, 4.80 (2H, AB q, J=13 Hz, H-20), 5.80 (1H, s, H-3), 6.15 (2H, m, H-1 and H-7), 7.1—7.8 (8H, complex pattern), 7.9—8.3 (2H, m), 8.85 (1H, d of d, J=1.0 and 10 Hz), 9.3—9.8, 11.6, and 13.3 (1H each, br s, OH) ppm	δ 0.93 (3H, d, J=6.0 Hz, H-18), 1.05 (3H, s), 1.09 (3H, s), 0.5—1.2 (2H, m), 1.76 (3H, d, J=1.0 Hz, H-19), 2.78 (6H, s), 3.65 (1H, m, H-5), 3.6—3.7 (1H, br s, OH), 4.10 (1H, m, H-8), 4.0—4.2 (2H, br s, OH), 4.35 (1H, s, H-3), 4.34, 4.83 (2H, AB q, J=13 Hz, H-20), 5.85 (1H, q, J=1.0 Hz, H-1), 6.12 (1H, m, H-7), 6.9—7.8 (8H, complex pattern), 7.9—8.2 (2H, m), 8.79 (1H, d, of d, J= 1.0 and 10 Hz), 9.50, 11.7, and 13.4 (1H each, br s, OH) ppm	δ 1.02 (3H, d, J=6.0 Hz, H-18), 1.03 (6H, s, H-16 and H-17), 1.80 (3H, d, J=1.0 Hz, H-19), 2.80 (6H, s), 3.65 (1H, s, OH), 3.8—4.5 (6H, m, H-5, H-8, H-20, and 2 OH), 5.75 (1H, s, H-3), 6.0—6.2 (2H, m, H-1 and H-7), 7.0—7.8 (8H, complex pattern), 7.9—8.3 (2H, m), 8.80 (1H, m), 9.5, 11.5, and 13.2 (1H each, br s, OH) ppm

groups was indicated by the IR spectrum (1670, 1630, 1510, and 1300 cm^{-1}). The remaining nitrogen atom was deduced to be present in a dimethylamino group, judging from the signal in the NMR spectrum of compound **4** [δ 2.73 (6H, s)]. Also, the remaining oxygen atom was suggested to be in a hydroxyl group forming strong a hydrogen bond, which was assigned to the signal at δ 12.9 in the NMR spectrum. This data suggests that compound **4** possesses aromatic rings. A high-resolution mass spectrum showed the presence of three fragment ions, $C_9H_{10}NO^{+\cdot}$, $C_7H_5NO_3^{+\cdot}$, and $C_8H_9NO_2^{+\cdot}$. This indicates that these fragments are dimethylanthraniloyl, hydroxyanthraniloyl, and methyl anthranilate groups, respectively. In particular, the dimethylanthraniloyl group was confirmed by the fact that oxidation of **4** with $KMnO_4$ afforded a quinazolone (**7**), which was identified with a naturally-occurring alkaloid (**7**), glycomicine,[3] from spectral data[4] and physical constants. This compound (**7**) was produced by the oxidation of one methyl group in the dimethylanthraniloyl group and the aromatic ring in the hydroxyanthraniloyl group, followed by cyclization. The hydrolysis of **4** with an aqueous NaOH solution afforded a carboxylic acid (**8**), but with 6 M HCl–MeOH gave methyl *N*-(dimethylanthraniloyl)-3-hydroxyanthranilate (**9**) and methyl anthranilate. Compound **9** was deduced to possess a dimethylanthraniloyl group and a hydroxyanthraniloyl from its fragmentation of mass spectrum. The fragment peak resulting from dehydration, in this spectrum, was much stronger than that of the usual phenolic compounds. This observation is explained by the next reaction: **9** was readily dehydrated to a benzoxazole (**10**) in a gas-liquid chromatograph. That the hydroxyanthraniloyl group is 3-hydroxyanthraniloyl, but not 6-hydroxyanthraniloyl (*i.e.* a 3-aminosalicyloyl group), was supported by a comparison of the NMR spectra of compound **9** and a 3-aminosalicyloyl derivative. The structure of compound **9** was confirmed by synthesis. Dimethylanthranilic acid was converted to the imidazolide by the action of *N,N'*-carbonyldiimidazole with no solvent. To a THF solution of this imidazolide was added 3-hydroxyanthranilic acid, and the subsequent addition of CH_2N_2 gave methyl *N*-(dimethylanthraniloyl)-3-hydroxyanthranilate, which exhibits IR, NMR, and mass spectra and a melting point identical with compound **9**.

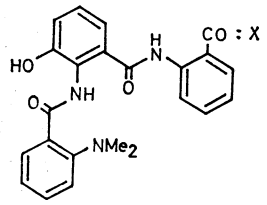
On the other hand, alcohol **5** was converted to a triacetate (**11**), which was identical with ingenol triacetate. The structure of ingenol triacetate was unambiguously established using X-ray analysis by Hecker *et al.*[5]

Milliamine A is an ingenol derivative esterified by acid **8**, as described above, and by acetic acid, the presence of the latter acid being apparent in the NMR spectrum (see Table 1). Although the esterified position of ingenol remains to be defined, this problem was solved by the following chemical and spectral evidence. Treatment of the hydrochloride of milliamine A with methanol–aqueous $NaHCO_3$ gave ingenol monoacetate (**12**), whose NMR spectrum showed that the signal due to two protons at C-20, which appeared at δ 4.10 in ingenol (**5**), was observed as an AB quartet at δ 4.25 and 4.80 (J=10 Hz), indicating that a primary acetoxyl group is present in milliamine A (**1**). Furthermore, in the 100 MHz NMR spectrum of milliamine A, the singlet observed at δ 5.80 (δ 4.32 in ingenol) was converted into a sharp singlet upon irradiation of the doublet attributed to three protons at C-19 [δ 1.83 (J=1.0 Hz)]. This phenomenon can be understood as long-range coupling through the sp^2 carbon atom. In addition, the fact that this signal, appearing at δ 5.80, can be attributed to the proton at C-3 was clarified by the following chemical evidence. Ingenol (**5**) was converted to a monocarbonate (**13**) by a reaction with *N,N'*-carbonyldiimidazole, which possesses a six-membered carbonate ring (IR 1745 cm^{-1}). The monocarbonate, **13**, gave a dicarbonate (**14**) using the same procedure at elevated temperatures, in which six-membered (1780 cm^{-1}) and five-membered (1830 cm^{-1}) carbonate rings were present. A comparison of the NMR spectra of carbonates **13** and **14** showed that only for the formation of the five-membered carbonate ring can the signal assigned to H-3 (δ 5.3) undergo a downfield shift. Moreover, cleavage of the bond between C-3 and C-4 in the monocarbonate (**13**) by $NaIO_4$ was attempted giving compound **15**: IR ($CHCl_3$) 3400, 1820, and 1725 cm^{-1}; MS *m/e* 372 (M^+); NMR (100 MHz, $CDCl_3$) δ 0.97 (3H, s), 1.08 (3H, s), 1.11 (3H, d, J=6.0 Hz, H-18), 1.90 (3H, br s, H-19), 2.80 (1H, m, H-11), 3.13 (1H, br d, J=10 Hz, H-8), 4.18, 4.50 (2H, AB q, J=13 Hz, H-20), 5.53 (1H, s, H-3), 5.82 (1H, br s, H-1), and 6.40 (1H, d, J=4.5 Hz, H-7). In the NMR spectrum of the urethane (**16**) of this compound **15**, the signal due to the protons at C-20 appears at δ 4.80 as a singlet. It is believed that this compound **15** was formed by aldol condensation of the desired keto-aldehyde intermediate (**17**) and subsequent migration of the carbonate group.

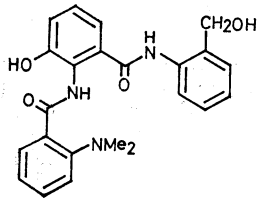
On the basis of these results, it is concluded that the



1 R_1=X, R_2=H, R_3=Ac
2 R_1=R_2=H, R_3=X
3 R_1=X, R_2=R_3=H
5 R_1=R_2=R_3=H
11 R_1=R_2=R_3=Ac
12 R_1=R_2=H, R_3=Ac



4 X=OCH_3
8 X=OH



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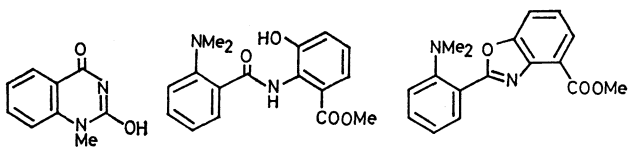
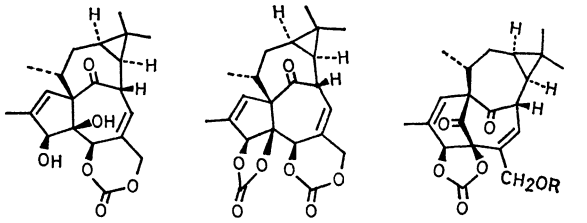
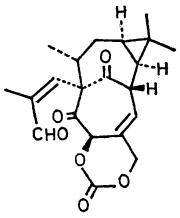


7 9 10



13 14 15 R=H 16 R=CONHPh



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structure of milliamine A is **1**.

Examination of the Acute Toxicity of Milliamine A. The acute toxicity of milliamine A was examined using 30 male ICR-SLC strain mice. The mode of administration was intraperitoneal injection. For one week after njection, LD_{50} was determined from the mortality rate of the mice according to Weil's method.[6] The LD_{50} of milliamine A was 0.642 mg/kg. The symptoms observed 2—3 h after the injection were slight ptosis, diarrhea, decreased respiration, decreased body temperature, tremors, *etc.*, and occasionally, dyspnoe and clonic-tonic convulsion. From autopsies of the mice, hemorrhaging and lung and spleen congestion were observed. It is assumed that the cause of death was respiratory arrest derived from injuries to the circulatory system. In a control experiment, 20% ethanol was used as a solvent, but no deaths were observed. These results are summarized in Table 2.

Table 2. Toxicity of milliamine A

Dose injected intraperitoneally (μg/kg)	Mortality	LD_{50} (μg/kg)
270	0/5	
400	1/5	
600	1/5	642
900	5/5	
1350	4/5	
2025	5/5	

The Structure of Milliamine B. In milliamine B (**2**) an acetoxyl group is present. The structure of milliamine B is suggested from the result that methanolysis of milliamine B afforded a compound (**4**) and an ingenol (**5**), which was identified as ingenol triacetate (**7**). The position esterified by the alkaloidal moiety was at C-20, on the basis of the NMR spectrum in which the signals attributed to two protons appeared at δ 4.43 and 4.83 as an AB quartet.

Thus, the structure of milliamine B is represented by **2**.

The Structure of Milliamine C. Milliamine C (**3**) afforded a compound **4** and ingenol (**5**) upon methanolysis with NaOMe in MeOH. The ingenol (**5**) was converted to triacetate **7** for identification with an authentic sample. In the NMR spectrum of **3**, the acetoxyl group observed in the NMR spectrum of milliamine A, was absent and the signal assigned to the proton at C-3 appeared at δ 5.75 as a singlet. Consequently, the structure of milliamine C was determined to be **3**.

Although the relationship between the toxicity and the structure is complex, the following facts are suggested. Ingenol and the triacetate are non-toxic, but ingenol derivative, in which the hydroxyl group at C-3 was esterified with fatty acids[2,7] or an alkaloidal moiety, indicate toxicity. However, according to our results, it appears that at least the acetylation of the hydroxyl group at C-20 has no relation to the toxicity of these derivatives because milliamines A and C are toxic. On the other hand, the presence of a 3-hydroxyanthraniloyl derivative is rare[8] and its carcinogenic properties may influence the cocarcinogenic characteristics of ingenol derivatives reported by Hecker.[7] During this work, experiments concerning the carcinogenic activity of milliamine A were undertaken, but no results were obtained due to interference of the strong toxicity.

Experimental

All melting points are uncorrected. The UV spectra were measured in MeOH on a Perkin-Elmer Model 202 spectrophotometer. The IR spectra were recorded with a Jasco Model IRS spectrometer. The NMR spectra were determined on JNMC-60H, JNM4H-100, and Varian HA-100 spectrometers; Chemical shifts (δ) are given in ppm relative to the internal TMS. The mass spectra were determined on a Hitachi RMU-6C mass spectrometer equipped with a direct inlet system, and the high-resolution mass spectra on a JEOLCO GMS-O1SG mass spectrometer. A Hitachi K-53 gas chromatograph was employed for analytical GLC. A Varian 1820-4 gas chromatograph was used for preparative GLC. For TLC, silica gel GF_{254}, PF_{254} (E. Merck, A. G., Germany) and alumina PF_{254}-Type T (E. Merck, A. G., Germany) were used; the thickness employed were 0.25 mm for analytical purposes, and 1.00 mm for preparative purposes. For column chromatography, silica gel (100 mesh, Mallinckrodt, U.S.A.) was used. The organic solutions were dried over Na_2SO_4 and evaporated in a vacuum evaporator.

Extraction of Milliamines A (1), B (2), and C (3). Roots (7.7 kg) of *Euphorbia Millii* Ch. des Moulins (45 kg) were dried and cut into small pieces. 20 l of methanol was added to the roots. The mixture was left for a few days at room temp and was filtered with suction. This methanol-extraction procedure was repeated twice. The combined methanol extracts were concentrated below 60 °C, to afford a green solution (50 ml) which was diluted with ether (500 ml). The ether layer was separated from a water layer which was extracted with ether three times. The combined ether

solutions were washed with saturated NaCl aq solution, dried, and concentrated, giving an oily mixture (41 g). The resulting material was subjected to chromatography on silicic acid (500 g) with $CHCl_3$. The eluates of $CHCl_3$ afforded some triterpene compounds. After changing the solvent to $CHCl_3$–MeOH (V/V=20/1), the early fraction gave an oily material (7 g) containing milliamine A. To an ethereal solution (100 ml) of this mixture was added 15 ml of ether saturated with HCl gas. Filtration with suction afforded a green powder of hydrochloride (1.2 g), which was dissolved in acetone (5 ml) and the solution was allowed to stand, giving crude crystals (691 mg). Recrystallization from acetone afforded 480 mg of milliamine A hydrochloride. The next eluate gave a mixture of milliamines B and C, which were purified by repeated TLC. Milliamine B (67 mg) was obtained as a hydrochloride following the same procedure as for milliamine A. The unstable properties of milliamine C in acidic conditions did not permit further purification *via* hydrochloride formation. After repeated TLC, 30 mg of milliamine C was obtained. The physical and spectral data are recorded in Table 1. **1**, (Found: C, 64.80; H, 5.99; N, 4.84; Cl, 4.30%. Calcd for $C_{45}H_{50}N_3O_{10}Cl$: C, 64.76; H, 6.10; N, 5.07; Cl, 4.28%). **2**, (Found: C, 64.53; H, 6.04; N, 5.19%. Calcd for $C_{43}H_{48}N_3O_9Cl$: C, 64.50; H, 6.09; N, 5.32%).

*Methanolysis of Milliamine A (**1**).* To a solution of milliamines A hydrochloride (30 mg) in anhydrous MeOH (2 ml) was added a solution (0.5 ml) of NaOMe in MeOH (prepared from 100 mg of Na and 20 ml of anhydrous MeOH) at room temp. The solution was stirred at room temp for 1 h. Neutralization through a column of Amberlite IRC-50 (H-form) (4 ml) was performed. This eluate and a methanol washing solution were combined and concentrated. The resulting yellow oily material was subjected to chromatography on silicic acid. The $CHCl_3$ eluate afforded compound **4** (17 mg), which was recrystallized from MeOH, and the 10% MeOH–$CHCl_3$ eluate gave glassy **5** (15 mg). **4**, Found: *m/e* 433.1638. Calcd for $C_{24}H_{23}N_3O_7$: *m/e* 433.1631. Found: *m/e* 151.0614. Calcd for $C_8H_9NO_2$: *m/e* 151.0633. Found: *m/e* 148.0747. Calcd for $C_9H_{10}NO$: *m/e* 148.0762. Found: *m/e* 135.0304. Calcd for $C_7H_5NO_2$: *m/e* 135.0304. **5**, IR ($CHCl_3$) 3410, 1705, and 1635 cm^{-1}; NMR (60 MHz, $CDCl_3$) 0.6—1.2 (2H, m, H-13 and H-14), 0.94 (3H, d, J=6.0 Hz, H-18), 1.12 (3H, s), 1.52 (3H, s), 1.82 (3H, d, J=1.0 Hz, H-19), 3.77 (1H, br s, H-5), 4.10 (2H, br s, H-20), 4.32 (1H, s, H-3), 4.0—4.4 (1H, m, H-8), 3.0—4.0 (4H, br s, OH), 5.84 (1H, q, J=1.0 Hz, H-1), and 6.02 (1H, br d, J=3.0 Hz, H-7); MS 348 (M^+).

*Alcohol (**6**).* A mixture of **4** (5 mg) and LAH (3 mg) in anhydrous ether (2 ml) was stirred at 0 °C for 1 h, and diluted with 5 ml of water. The mixture was extracted with three 5 ml portions of $CHCl_3$. The combined $CHCl_3$ layers were washed with saturated NaCl aq solution, dried, and concentrated to afford an oily product. The product was purified by preparative TLC. **6**, IR ($CHCl_3$) 3600, 3550, 1655, 1630, 1595, 1515, and 1315 cm^{-1}; NMR (60 MHz, acetone-d_6) 2.72 (6H, s), 4.70 (2H, br s,) 4.70 (1H, s, OH), 7.0—7.5 (10H, m), 8.00 (1H, m), 9.42, 9.80, and 13.2 (1H each, br s, OH); MS 405 (M^+).

*Quinazolone (**7**).* A mixture of **4** (37 mg) and $KMnO_4$ (40 mg) in a 6% aq NaOH solution was stirred at room temp for 15 min. After the addition of water (5 ml), the aq solution was repeatedly extracted with $CHCl_3$. The $CHCl_3$ layer were dried over Na_2SO_4, and concentrated giving an oily material (10 mg). The water layer was neutralized with HCl and extracted with $CHCl_3$ (5 ml). The extracts were dried over Na_2SO_4 and concentrated to give compound **7**. Recrystallization from ether afforded crystalline **7** (3 mg); mp 269—270 °C (lit, 269—270 °C);[3] IR (KBr) 3410, 1708, 1690, 1660, and 1605 cm^{-1}; MS 176 (M^+) (Found: *m/e* 176.0585. Calcd for $C_9H_8N_2O_2$: *m/e* 176.0587).

*Hydrolysis of **4**.* Compound **4** (5 mg) was dissolved in MeOH (2 ml). After the addition of a 5% aq NaOH solution (2 ml) to the solution, the mixture was stirred at room temp for 2 h. After acidification (to *ca.* pH 2) with an aq HCl solution, a cloudy solution was extracted with three 3-ml portions of $CHCl_3$. The $CHCl_3$ layers were combined, dried over Na_2SO_4, and concentrated to give an oily compound **8** (3 mg); IR ($CHCl_3$) 2800—3500, 1690, 1670, 1625, and 1590 cm^{-1}; NMR (60 MHz, $CDCl_3$) 2.90 (6H, s), 7.1—7.6 (8H, m), 8.0—8.3 (3H, m), and 9.5—10.0 (4H, br s, OH) (Found: *m/e* 419.1500. Calcd for $C_{23}H_{21}N_3O_5$: *m/e* 419.1506).

*Methyl N-(Dimethylanthraniloyl)anthranilate (**9**).* A solution of compound **4** in MeOH (2 ml)–6 M HCl (2 ml) was stirred at 60 °C for 18 h. After dilution with 10 ml of water, the mixture was extracted with five 5-ml potions of $CHCl_3$. The extracts were dried over Na_2SO_4 and concentrated to give an oily product (15 mg). Purification by preparative TLC on alumina with benzene yielded **9** (7 mg) and methyl anthranilate (2 mg), **9**, mp 89—90 °C (recrystallized from methanol); IR ($CHCl_3$) 2800—3450, 1705, 1630, 1595, and 1300 cm^{-1}; NMR (60 MHz, acetone-d_6) 2.84 (6H, s), 3.88 (3H, s), 7.0—7.7 (6H, m), 8.10 (1H, m), 9.43, and 13.1 (1H each, br s,); MS 314 (M^+), 296 (Found: *m/e* 314.1266. Calcd for $C_{17}H_{18}N_2O_4$: *m/e* 314.1283).

*Benzoxazole **10**.* Injection of compound **9** (10 mg) into a column (5% SE-30 on chromosorb W, 1′/4×10 ft) for GLC (270 °C, 60 ml/h flow of He carrier gas) afforded **10** after a period of 4.2 min. **10**, IR ($CHCl_3$) 1715, 1600, 1570, 1540, 1490, and 1300 cm^{-1}; NMR (60 MHz, acetone-d_6) 2.76 (6H, s), 3.90 (3H, s), and 6.8—8.0 (7H, m) (Found: *m/e* 296.1199. Calcd for $C_{17}H_{16}H_2O_3$: *m/e* 296.1190).

*The Synthesis of Methyl N-(Dimethylanthraniloyl)anthranilate (**9**).* A mixture of methyl dimethylanthranilate (50 mg) and *N,N′*-carbonyldiimidazole (50 mg) was heated to 90 °C for 2 min and maintained at room temp for 30 min. To the mixture, 50 mg of 3-hydroxyanthranilic acid was added. This oily mixture was maintained at 90 °C for 10 min, and was dissolved in a 30-ml solution of THF and stirred at 90 °C for 2 h. After dilution with water (10 ml), the solution was extracted with five 10-ml portions of $CHCl_3$. The extracts were dried over Na_2SO_4 and concentrated to afforded an oily mixture. The oily product was methylated in ether with CH_2N_2, giving crude **9**. The mixture was separated by preparative TLC on alumina with benzene. The eluate from alumina with $CHCl_3$–AcOEt (1: 1) which was then evaporated afforded **9** (72 mg), which was recrystallized from methanol to give **9** (55 mg); mp 89—90 °C.

*Ingenol Triacetate (**11**).* A solution of ingenol (**5**) (32 mg) in Ac_2O (0.06 ml) and dried pyridine (2 ml) was maintained at room temp overnight, to which 10 ml of water was added. The solution was extracted with five 10-ml portions of $CHCl_3$. The $CHCl_3$ layers were combined, washed with an aq HCl solution, an aq $NaHCO_3$ solution, and saturated NaCl aq solution dried over Na_2SO_4, and concentrated to give an oily compound. This oily product was recrystallized from MeOH to afford crystalline ingenol triacetate (**11**) (25 mg); mp 197—198 °C (lit, 196—197 °C);[5] IR ($CHCl_3$) 3440, 1740, 1710, and 1635 cm^{-1}; NMR (60 MHz, $CDCl_3$) 0.6—1.0 (2H, m), 1.00 (3H, d, J=6.0 Hz), 1.07 (3H, s), 1.11 (3H, s), 1.78 (3H, d, J=1.5 Hz), 2.03 (3H, s), 2.15 (3H, s), 2.23 (3H, s), 3.25 (1H, s, OH), 4.0—4.3 (1H, m), 4.18, 4.62 (2H, AB q, J=12 Hz), 5.00 (1H, s), 5.41 (1H, br s), 6.10 (1H, q, J=1.0 Hz), and 6.25 (1H, br d, J=4.5 Hz) (Found: *m/e*

474.2259. Calcd for $C_{26}H_{34}O_8$: *m/e* 474.2253).

Ingenol Monoacetate (12). Milliamine A hydrochloride (52 mg) was dissolved in an aq solution (10 ml) of $NaHCO_3$ (300 mg) and 20 ml of MeOH. This mixture was stirred for 1 h at room temp. Water was added to this solution, which was extracted with five 10-ml portions of $CHCl_3$. The $CHCl_3$ layers were combined, washed with a saturated NaCl solution, dried over Na_2SO_4, and concentrated to give an oily product (55 mg). This product was purified by preparative TLC to give ingenol monoacetate (**12**) (15 mg); IR ($CHCl_3$) 3480, 1725, and 1645 cm^{-1}; NMR (60 MHz, $CDCl_3$) 0.95 (3H, d, J=6.0 Hz), 1.03 (3H, s), 1.07 (3H, s), 1.80 (3H, d, J=1.0 Hz), 2.01 (3H, s), 3.60 (1H, br s), 3.8—4.2 (1H, m), 4.40 (1H, s), 4.25, 4.80 (2H, AB q, J=10 Hz), 5.90 (1H, q, J=1.0 Hz), and 6.05 (1H, m) (Found: *m/e* 390.2014. Calcd for $C_{22}H_{30}O_6$: *m/e* 390.2041).

Ingenol Monocarbonate (13). A mixture of ingenol (**5**) (20 mg) and *N,N'*-carbonyldiimidazole (15 mg) was dissolved into THF (2 ml), stirred for 2 h at room temp and monitered by TLC on silicic acid. Evaporation of THF at 40 °C afforded an oily product, which was dissolved into a 1% aq HCl solution (5 ml) and $CHCl_3$ (5 ml) by shaking. The $CHCl_3$ was separated and purified by preparative TLC to give monocarbonate **13** (13 mg) and ingenol (5 mg) **13**, IR ($CHCl_3$) 3450, 1745, 1725, and 1665 cm^{-1}; NMR (60 MHz, $CDCl_3$) 0.90 (3H, d, J=6.0 Hz), 1.05 (3H, s), 1.10 (3H, s), 1.83 (3H, d, J=1.0 Hz), 4.0—5.0 (7H, m), 5.80 (1H, q, J=1.0 Hz), and 6.20 (1H, m); MS 374 (M^+).

Ingenol Dicarbonate (14). A THF solution (1 ml) of monocarbonate (**13**) (10 mg) and *N,N'*-carbonyldiimidazole (10 mg) was stirred at 40 °C for 2 h and concentrated to give a mixture. After preparative TLC, an oily product was recrystallized from ether to afford crystalline **14**; mp 204—206 °C; IR ($CHCl_3$) 1830, 1780, 1740, and 1660 cm^{-1}; NMR (60 MHz, $CDCl_3$) 0.9 (3H, d, J=6.0 Hz), 1.1 (3H, s), 1.2 (3H, s), 2.1 (3H, d, J=1.0 Hz), 3.7 (1H, br. d, J=12 Hz), 4.5—4.8 (2H, AB q, J=13.5 Hz), 4.7 (1H, m), 5.3 (1H, s), 5.9 (1H, q, J=1.0 Hz), and 6.2 (1H, m) (Found: *m/e* 400.1510. Calcd for $C_{22}H_{24}O_7$: *m/e* 400.1521).

Compound 15. A mixture of monocarbonate **13** and an aq $NaIO_4$ solution (100 mg/5 ml H_2O) in THF was stirred for 7 h at 40 °C. After the addition of water (5 ml), the mixture was extracted with four 5-ml portions of $CHCl_3$. The extracts were washed with saturated NaCl aq solution, dried, and concentrated to give oily **15** (13 mg) (Found: *m/e* 372.1518. Calcd for $C_{21}H_{24}O_6$: *m/e* 372.1571).

Urethane 16. A mixture of compound 15 (10 mg) and phenyl isocyanate (30 mg) in THF containing a trace of pyridine was stirred at 80 °C for 3 h. After dilution with water, the aq solution was extracted with three 5-ml portions of $CHCl_3$. The extracted layers were combined, dried over Na_2SO_4, and concentrated to yield a product. Separation and purification by preparative TLC on silicic acid afforded glassy **16**; IR ($CHCl_3$) 3430, 1830, 1730, 1605, and 1525 cm^{-1}; NMR (60 MHz, $CDCl_3$) 4.80 (2H, s), 5.50 (1H, q, J=1.0 Hz), 5.95 (1H, m), 6.50 (1H, br d, J=4.5 Hz), 6.90 (1H, s, NH), and 7.00 (5H, m); MS 491 (M^+).

Methanolysis of Milliamine B (2). To a solution of **2** (29 mg) and anhydrous MeOH (2 ml) was added a solution (0.3 ml) of NaOMe in MeOH (prepared from 374 mg of Na and 40 ml of anhydrous MeOH). The solution was maintained at room temp for 1 h and was added the Amberlite IRC-50 (H-form) required for neutralization of the NaOMe. The resin was filtered with suction and the filtrate was concentrated, affording an oily mixture (32 mg), which was separated by preparative TLC on silica gel. Two eluates of AcOEt were concentrated, affording **4** and **5.** Compound **4** was recrystallized from methanol to give crystalline **4** (7 mg); mp 160—162 °C. Ingenol (**5**) was acetylated with Ac_2O and pyridine (2 ml) to give ingenol triacetate (**11**). **11**, mp 196—198 °C (recrystallized from MeOH).

Methanolysis of Milliamine C (3). To a solution of milliamine C (22 mg) in anhydrous MeOH (2 ml) was added a solution (0.1 ml) of NaOMe in MeOH (prepared from 200 mg of Na and 40 ml of anhydrous MeOH). The solution was maintained at room temp for 1 h and the Amberlite IRC-50 (H-form) required for neutralization of the NaOMe. The resin was filtered with suction and the filtrate was concentrated, affording an oily mixture (18 mg). The mixture was separated by preparative TLC on silica gel. Two eluates of AcOEt were concentrated to yield **4** and **5.** Compound **4** was recrystallized from MeOH to afford crystalline **4** (2 mg); mp 159—161 °C. Alcohol **5** was treated with Ac_2O (0.1 ml) and pyridine (2 ml), followed repeated by evaporation of the Ac_2O and pyridine under reduced pressure after addition of benzene. The residue was recrystallized from MeOH to give crystalline **11**, mp 195—198 °C.

References

1) D. Uemura and Y. Hirata, *Tetrahedron Lett.*, **1971**, 3673.
2) D. Uemura and Y. Hirata, *Tetrahedron Lett.*, **1973**, 881.
3) S. C. Pakrashi and H. Budzikiewicz, *Tetrahedron*, **19**, 1011 (1963).
4) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products of Mass Spectrometry," Vol. I, Holden-Day, Inc., San Francisco (1964), p. 212.
5) K. Zechmeister, F. Brandl, W. Hoppe, E. Hecker, H. J. Opferkuch, and W. Adolf, *Tetrahedron Lett.*, **1970**, 4075.
6) C. S. Weil, *Biometrics*, **8**, 249 (1952).
7) E. Hecker, *Cancer Res.*, **28**, 2338 (1968).
8) A. J. Evans, *J. Chem. Soc.*, **101**, 544 (1912).