DDQ OXIDATION OF RING-A METHOXYLATED 11b-METHOXYCARBONYL-2,3,5,6,11,11b-HEXAHYDRO-3-OXO-1*H*-INDOLIZINO[8,7-*b*]INDOLES

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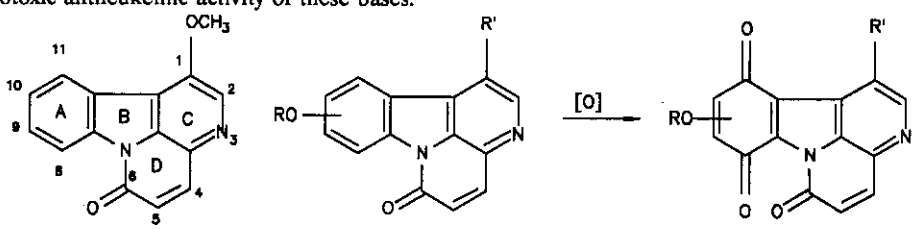
Abstract- A study of the oxidation of ring-A methoxylated 11b-methoxycarbonyl-2,3,5,6,11,11b-hexahydro-3-oxo-1*H*-indolizino[8,7-*b*]indoles (**8a** - **8e**) with DDQ in aqueous tetrahydrofuran was carried out. Treatment of **8a** - **8c** under these conditions effected regiospecific oxidation at C–6 in yields ranging from 40-73% to provide the corresponding ring-A methoxylated 3,6-dioxo-1*H*-indolizino[8,7-*b*]indoles (**12a** - **12c**), respectively. The oxidation of the 7,8,9-trimethoxy analog (**8d**) also gave the 6-oxo derivative (**12d**), albeit in low yield. In contrast, the reaction of 8,10-dimethoxyindolizino[8,7-*b*]indole (**8e**) with DDQ (aq. THF) generated the quinone (**12e**), the structure of which is reminescent of the mitomycins. A proposed pathway for the origin of quinone (**12e**) is presented.

Recently, the synthesis of the cytotoxic antileukemic alkaloid 1-methoxycanthin-6-one (**1**) was reported.[1] This alkaloid and related congeners exhibit cytotoxic, antileukemic activity *via* their inhibitory effects on DNA synthesis in GPK epithelial cells.[2]

It is known that oxygenation of the canthin-6-one skeleton at position-1 or in ring-A enhances the cytotoxic antileukemic activity of these bases.[2]

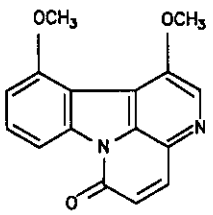


1 **2a**, R′ = OCH_3 **3a**, R′ = OCH_3
2b, R′ = H **3b**. R′ = H

It is possible that these planar canthin-6-one alkaloids may undergo oxidation of ring-A *in vivo*,

analogous to the oxidation of the serotonergic neurotoxin 5,7-dihydroxytryptamine,[3] to provide quinone intermediates (see **2** → **3**) which elicit the cytotoxic activity. On the other hand, these alkaloids may demonstrate the multimodal mechanism of action which is exhibited by the planar antitumor drugs in the ellipticine series.[4,5] This is especially important in regard to the activity of 2-methyl-9-hydroxyellipticinium acetate, because of its known therapeutic use and the proposed involvement of a quinone-imine in the mechanism of antitumor action.[4,5]

Efforts recently have centered on an approach to the synthesis of ring-A substituted canthin-6-ones both in the natural (see **4**) and unnatural series (see **5**) in order to evaluate the importance of ring-A



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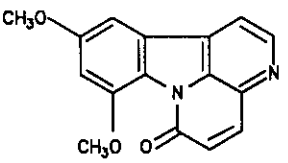

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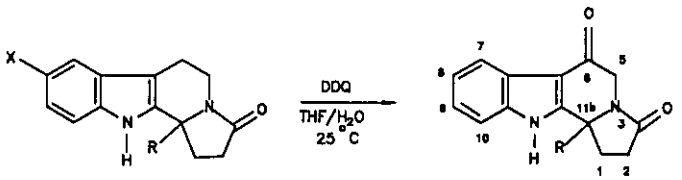
on cytotoxic activity. The crucial step employed in the previous synthesis of alkaloid (**1**) was based on the regiospecific dichlorodicyanoquinone (DDQ) oxidation (THF/H_2O)[6] of an 11b-methoxycarbonyl substituted [8,7-*b*]indolizinoindole (**6**) to provide the 6-oxo-[8,7-*b*]indole (**7**),[6,7] as illustrated below.

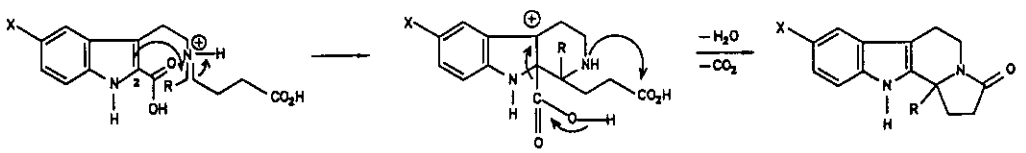
Scheme 1



6, R = CO_2CH_3 **7**, R = CO_2CH_3

The construction of the required ring-A substituted methyl (alkoxy)-2,3,5,6,11,11b-hexahydro-3-oxo[8,7-*b*]indolizinoindole-11b-carboxylates (**8a** - **8e**) was accomplished either by the Moody azide

Scheme 2



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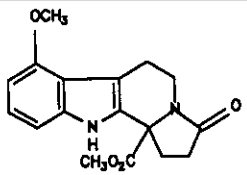
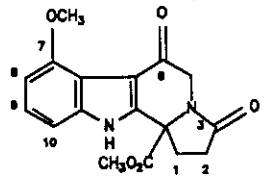
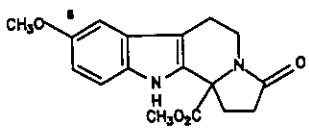
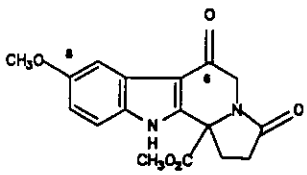
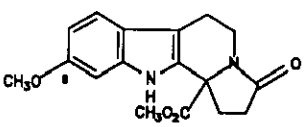
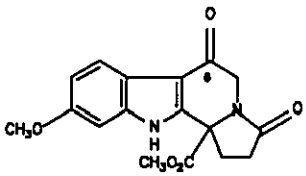
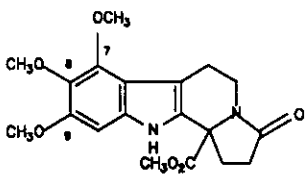
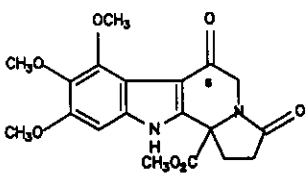
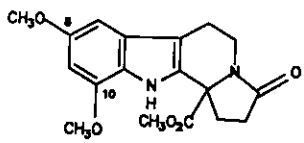
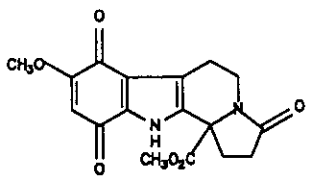
pyrolysis/gramine protocol (**8a** and **8c**)[7] or by the carboxyl-mediated Pictet-Spengler reaction (**8b**, **8d** and **8e**).[8,9] The proposed pathway for the latter process is outlined in Scheme 2. The details of this chemistry are described in references 7-9 and need not be repeated here.

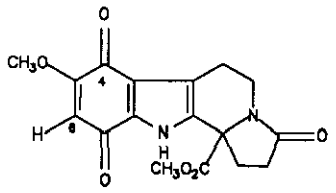
Table I: DDQ oxidation of methoxy substituted 11b-carbomethoxy-2,3,5,6,11,11b-hexahydro-3-oxo-1*H*-indolizino[8,7-*b*]indoles.

Entry	Substrate	Product (yield %)	
1.	 **8a**	 **12a**	(40)[a]
2.	 **8b**	 **12b**	(73)
3.	 **8c**	 **12c**	(65)
4.	 **8d**	 **12d**	(21)[a]
5.	 **8e**	 **12e**	(25)[a]

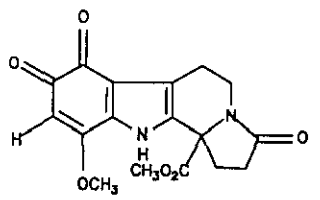
[a]These yields have not been maximized to date. Additional material remains in the mother liquor.

In order to extend the DDQ technology to the synthesis of ring-A oxygenated canthin-6-ones, the methoxy substituted [8,7-*b*]indolizinoindoles (**8a** - **8e**) were treated with DDQ in aqueous THF at 25°C.[1] The results are summarized in Table 1. The three methoxy substituted analogs (**8a**), (**8b**) and (**8c**) furnished the desired 6-oxo-[8,7-*b*]indolizinoindoles (**12a, 12b, 12c**) respectively, in moderate yields (40 -73%). Moreover, the 7,8,9-trimethoxyindolizinoindole (**8d**) gave 6-oxo analog (**12d**) on treatment with DDQ, while the 8,10-dimethoxy derivative furnished the quinone (**12e**) when oxidized under the same conditions. The structure of quinone (**12e**) is reminiscent of those of mitomycins. The structure of **12e** was determined by comparison of the spectral data with that of quinones in the mitomycin series,[3,10] as illustrated here. The proton chemical shift for H-6 in the proton spectrum of **12e** is similar to that in the mitomycin derivative (**12g**).[10a] More importantly, the carbon-13 nmr chemical shifts for carbon atoms (4, 6, and 7) in **12e** are almost identical to the corresponding signals in mitomycin analog (**12g**) (see Figure 1). These signals are much different from those observed for the orthoquinone (**12h**)[10b] (see Figure 1) and suggest that paraquinone (**12e**) is the correct structure rather than orthoquinone (**12f**).

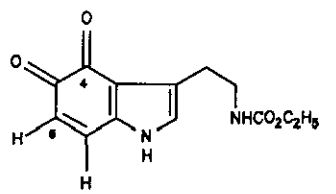
Figure 1



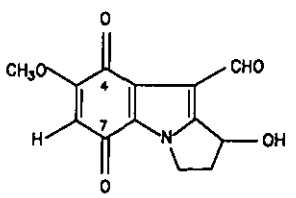
12e, ^{1}H nmr ($CDCl_3$) δ 5.65 (H-6)* ^{13}C nmr ($CDCl_3$) δ 105.78 (C-6), 177.42 (C-4), 178.18 (C-7) uv-vis (pH 7.4) λ_{max} 450, 340, 285, 235 nm; pH = 2 (no shift)



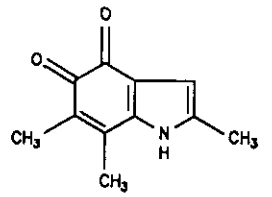
12f



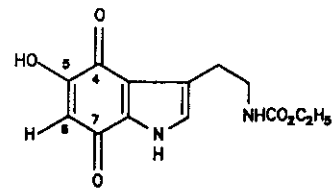
12h, ^{1}H nmr (DMSO-d_6) δ 5.88 (H-6) ^{13}C nmr ($CDCl_3$) δ 123.2 (C-6), (C-6) 173.9 (C-4), 184.0 (C-5)[10b] uv-vis (MeOH) λ_{max} 542, 353, 234 nm



12g, ^{1}H nmr ($CDCl_3$)[10a] δ 5.75 (H-6)* ^{13}C nmr ($CDCl_3$) δ 105.90 (C-6), 178.2 (C-4), 178.1 (C-7)



12i, uv-vis (CH_3OH) λ_{max} 568, 337, 242 nm[10c]



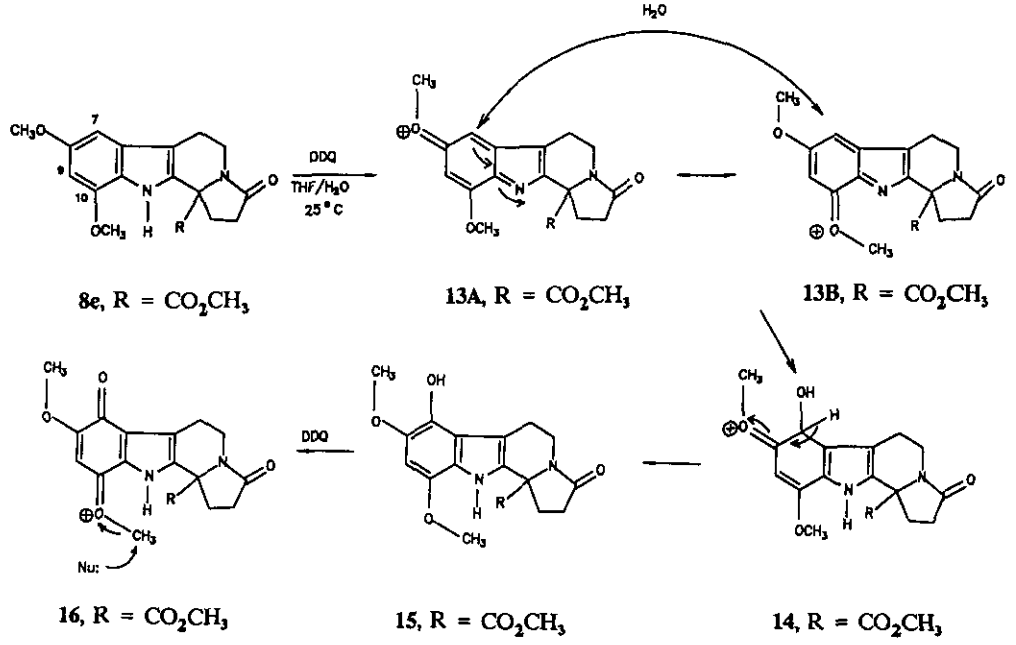
12j, ^{1}H nmr (DMSO-d_6) δ 5.64 (H-6)[3] ^{13}C nmr (DMSO-d_6) δ 106.27 (C-6), 178.75 (C-4), 178.50 (C-7) uv-vis [0.05 M phosphate buffer, (pH 7.4)] λ_{max} 527, 302, 235 nm; (pH 2) 466, 339, 286, 226 nm

Furthermore, the values of λ_{max} in the uv-visible spectrum of paraquinone (**12e**) are similar to those of paraquinone (**12j**) (in the protonated phenolic form, pH 2), but much different from the absorbances of either orthoquinone (**12h**)[10b] or (**12i**).[10c] Taken together this evidence supports the structure of paraquinone (**12e**), as reported rather than the alternative **12f**. Additional support for this assignment can be gleaned from the assignments for the model compounds (**12h-12j**) cited in references 3, 10, 11 and 12.

As shown in Table I, the oxidation of methoxy substituted [8,7-*b*]indolizinoindoles (**8a - 8d**) with DDQ is not exceptional and occurs with the same regiocontrol as the parent,[1] albeit the yields from Entries 1 and 4 are low. More importantly, the oxidation of the 8,10-dimethoxy analog (**8e**) furnished quinone (**12e**). This suggests that analogs related to **5** may exhibit enhanced cytotoxic activity *in vivo* in comparison to natural products such as **2** and **4**.

The mechanism for the formation of the 6-oxo-[8,7-*b*]indolizinoindoles (**12a, 12b, 12c** and **12d**) follows that earlier reported by Oikawa and Yonemitsu.[6] A proposed pathway for the conversion of **8e** into **12e** is outlined in Scheme 3. It is believed a charge transfer complex[6] forms between **8e** and DDQ to provide the quinone-imine intermediate (**13A**). The charge on oxygen can be equally distributed

Scheme 3



between mesomeric structures (**13A**)and (**13B**) facilitating attack of water at C-7 of ring-A to provide **14**, as illustrated. Loss of a proton from the monol (**14**) would generate the 8,10-dimethoxyphenol (**15**) which is oxidized by DDQ to quinone (**12e**) *via* the intermediate oxonium species (**16**). This is similar to the oxidation of aromatic compounds which contain methoxyl and hydroxyl groups disposed para to each other as reported in the literature.[10-12]

The preparation of the 3,6-dioxo[8,7-*b*]indolizinoindoles (**12a - 12d**) provides access to intermediates required for the synthesis of a number of natural and unnatural canthin-6-ones[1,2] bearing oxygen functionality in ring-A. The DDQ-mediated conversion of the 8,10-dimethoxy analog (**8e**) into quinone (**12e**) provides evidence that the enhanced cytotoxic activity of ring-A oxygenated canthin-6-ones may originate from quinone-imine[3-5] intermediates related to **13A** and **13B** or to quinone (**12e**). The similarity between **12e** and the mitomycins[10] is worthy of note.

EXPERIMENTAL

Microanalyses were performed on a F and M Scientific Corp. model 185 carbon, hydrogen, and nitrogen analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are reported uncorrected. Proton nmr spectra and ^{13}C nmr spectra were recorded on a Bruker 250 MHz spectrometer. Infrared spectra were taken on a Beckman Acculab-1 instrument, a Mattson Polaris R-10400, or a Nicolet Dx, while mass spectral data were obtained on a Hewlett Packard 5855 GC-mass spectrometer.

All chemicals were purchased from Aldrich Chemical Co. unless otherwise stated. Analytical tlc plates used were E. Merck Brinkmann uv-active silica gel. Silica gel 60b for column chromatography was purchased from E. M. Laboratories. The tlc plates were visualized under uv light or developed with spray reagents. The 1,2,3,4-tetrahydro-ß-carbolines were visualized by using a standard solution of ceric ammonium sulfate in 50% sulfuric acid. The synthesis of indolizino[8,7-*b*]indoles (**8a-8e**) was carried out as reported in reference 9.

In a typical procedure the indolizinoindole (1 mmol) and DDQ (4 mmol) were admixed and placed in a flask under a nitrogen atmosphere. The solution of THF/H_2O (10 ml, 9:1) was added and the

reaction mixture which took on a blue coloration was stirred at room temperature for 24 h. Ethyl acetate and aqueous saturated sodium bicarbonate solution were added to the red solution and the layers were separated. The aqueous layer was extracted with ethyl acetate and the combined organic layer was dried over sodium sulfate and passed through a column of neutral alumina. The solvent was removed under reduced pressure. Ether was added to precipitate the 6-oxo-[8,7-*b*]indolizinoindole which was filtered from the medium and dried.

Methyl 8-methoxy-2,3,5,6,11,11b-hexahydro-3,6-dioxo-1*H*-indolizino[8,7-*b*]indole-11b-carboxylate 12b: mp 238-239°C (EtOAc/Et_2O); ^1H nmr (DMSO-d_6) δ 2.20-2.80 (m, 4H), 3.70 (s, 3H), 3.75 (s, 3H), 3.80 (d, J = 18.0 Hz, 1H), 4.40 (d, J = 18.0 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 7.45 (m, 2H), 12.20 (s, 1H); ir (KBr) 3170, 1736, 1700, 1644, 1469, 1244, 1180 cm^{-1}; ms, CI(CH_4) (m/z) 329 (MH^+). Anal. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.20; H, 4.88. Found: C, 62.46; H, 4.95.

Methyl 7,8,9-trimethoxy-2,3,5,6,11,11b-hexahydro-3,6-dioxo-1*H*-indolizino[8,7-*b*]indole-11b-carboxylate 12d: mp 244-245°C (EtOAc/Et_2O); ^1H nmr ($CDCl_3$) δ 2.40-3.10 (m, 4H), 3.85 (s, 3H), 3.90 (s, 6H), 4.10 (s, 3H), 3.80 (d, J = 17.0 Hz, 1H), 4.90 (d, J = 17.0 Hz, 1H), 6.70 (s, 1H), 9.30 (br s, 1H); ir (KBr) 3200, 3000, 1750, 1675, 1650, 1450 cm^{-1}; ms, CI(CH_4) (m/z) 389 (M+1). Anal. Calcd for $C_{19}H_{20}N_2O_7$: C, 58.76; H, 5.15. Found: C, 58.61; H, 5.16.

Methyl 7-methoxy-2,3,5,6,11,11b-hexahydro-3,6-dioxo-1*H*-indolizino[8,7-*b*]indole-11b-carboxylate 12a: The amido ester (**8a**) (628 mg, 2 mmol) and DDQ (1.00 g, 4 mmol) were mixed together under a N_2 atm. THF (10 ml) was added followed by a mixture of THF/H_2O (8:2, 10 ml). The reaction mixture was stirred for 24 h. DDQ (1 equivalent) was added at 6 and 12 h intervals. The reaction mixture was diluted with EtOAc and extracted with aqueous $NaHCO_3$ (satd. solution). The EtOAc layer was dried over Na_2SO_4 and passed through a column of alumina (neutral). Evaporation of the ethyl acetate provided the target **12a** (164 mg, 25%): mp 130-132°C (EtOAc/Et_2O); ^1H nmr ($CDCl_3$) δ 2.30-2.70 (m, 4H), 3.70 (s, 3H), 3.85 (s, 3H), 3.95 (d, J = 18.0 Hz, 1H), 4.75 (d, J = 18.0 Hz, 1H), 6.60 (d, J = 8.0 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 11.20 (br s, 1H); ir (KBr) 3240, 2945, 1750, 1673, 1595 cm^{-1}; ms, CI(CH_4) (m/z) 329 (MH^+). Anal. Calcd for $C_{17}H_{16}N_2O_5 \cdot 0.25H_2O$: C, 61.35; H, 5.11. Found: C, 61.69; H, 5.31. The yield in this reaction can be increased to

40%, if one begins initially with 4-equivalents of DDQ.

Methyl 9-methoxy-2,3,5,6,11,11b-hexahydro-3,6-dioxo-1*H*-indolizino[8,7-*b*]indole-11b-carboxylate 12c: The γ-lactam (**8c**) (0.79 g, 2.5 mmol) and DDQ (1.70 g, 7.5 mmol) were mixed together and cooled to 0°C. A solution of THF/H_2O (20 ml, 9:1) was added dropwise. The blue-colored reaction mixture was warmed to room temperature. After 6 h another equivalent of DDQ was added and the reaction mixture was stirred overnight. Ethyl acetate and saturated $NaHCO_3$ were added and the layers were separated. The aqueous layer was extracted with EtOAc and the combined organic layer was dried over Na_2SO_4 and passed through a column of neutral alumina. The ethyl acetate was removed under reduced pressure. Ether was added to precipitate the product, which was filtered and dried to provide **12c**. (530 mg, 65%): mp 240-242°C (EtOAc/Et_2O); ^{1}H nmr (DMSO-d_6) δ 2.49 - 2.70 (m, 4H), 3.75 (s, 3H), 3.80 (s, 3H), 3.85 (d, J = 18.0 Hz, 1H), 4.45 (d, J = 18.0 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 7.00 (br s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 10.30 (s, 1H); ^{13}C nmr (DMSO-d_6) δ 29.04, 29.41, 47.06, 53.39, 55.34, 64.49, 95.90, 109.66, 111.99, 117.10, 121.16, 137.54, 146.36, 157.01, 169.83, 172.72, 186.18; ir (KBr) 3180, 1750, 1690, 1650, 1450, 1235, 1160 cm^{-1}; ms, CI(CH_4) (m/z) 329 (MH^+, 100%). Anal. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.20; H, 4.88. Found: C, 62.53; H, 5.06.

Methyl 8-methoxy-7,10-dioxo-2,3,5,6,11,11b-hexahydro-3-oxo-1*H*-indolizino[8,7-*b*]indole-11b-carboxylate 12e: The amido ester (**8e**) (344 mg, 1 mmol) and DDQ (0.90 g, 4 mmol) were mixed together and placed in a round bottom flask. A mixture of tetrahydrofuran/water (10 ml, 9:1) was added dropwise and the bluish-green colored solution was allowed to stir for one day at room temperature. Aqueous saturated sodium bicarbonate solution was extracted with $CHCl_3$. The combined chloroform layers were dried (Na_2SO_4) and evaporated under reduced pressure to provide the paraquinone (**12e**, 68 mg): mp 185-186°C (EtOAc/Et_2O); ^{1}H nmr ($CDCl_3$) δ 2.20-3.10 (m, 8H), 3.80 (s, 3H), 3.85 (s, 3H), 4.50 (m, 1H), 5.65 (s, 1H), 10.00 (s, 1H); On addition of D_2O the signals at δ 1.50 and 10.00 disappeared; ^{13}C nmr ($CDCl_3$) δ 21.89, 30.31, 31.01, 35.77, 53.32, 56.52, 64.82, 105.78, 118.35, 119.83, 131.70, 132.10, 160.81, 171.27, 173.07, 177.42, 178.18; ir (KBr) 3445, 3220, 2945, 1743, 1673, 1630, 1580, 1413, 1237, 1025 cm^{-1}; uv-visible spectrum λ_{max} (0.05 M, phosphate buffer pH 7.4) 450, 340, 285, 235 nm. There was no shift in values at pH 2.0; ms, CI(CH_4) (m/z) 345 (100), 346 (37.6), 347 (62.7), 348 (12.5). Anal. Calcd for $C_{17}H_{16}N_2O_6 \cdot H_2O$: C, 56.35; H, 4.97. Found: C, 56.33; H, 5.29. The

yellow-colored crystals of **12e** are characteristic of the structure of a p-quinone (see reference 11 for details).

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REFERENCES

1. T. J. Hagen, K. Narayanan, J. Names, and J. M. Cook, J. Org. Chem., 1989, **54**, 2170.
2. a) N. Fukamiya, M. Okano, and T. Aratani, J. Nat. Prod., 1986, **49**, 428. b) L. A. Anderson, A. Harris, and J. D. Phillipson, J. Nat. Prod., 1983, **46**, 374.
3. A. K. Sinhababu and R. T. Borchardt, J. Am. Chem. Soc., 1989, **111**, 2230. See also T. Tabatabaie, M. Z. Wrona, and G. Dryhurst, J. Med. Chem., 1990, **33**, 667. S. Singh and G. Dryhurst, J. Med. Chem., 1990, **33**, 3035.
4. For a review of the antitumor activity of the ellipticine series see C. Auclair, Arch. Biochem. Biophys., 1987, **259**, 1.
5. R. J. Hall, A. H. Jackson, A. M. F. Oliveira-Campos, M. J. Queiraz, and P. V. R. Shannon, Heterocycles, 1990, **31**, 401 and references cited therein.
6. Y. Oikawa and O. Yonemitsu, J. Org. Chem., 1977, **42**, 1213. M. Cain, R. Mantei, and J. M. Cook, J. Org. Chem., 1982, **47**, 4933.
7. C. J. Moody, J. Chem. Soc., Perkin Trans. 1, 1984, 1333. L. Henn, M. B. Hickey, C. J. Moody, and C. J. Rees, J. Chem. Soc., Perkin Trans. 1, 1984, 2189. R. J. Sundberg, 'The Chemistry of Indoles', Academic Press, New York, 1970.
8. The tryptamine-2-carboxylic acids (**9-11**) were prepared by the method of Abramovitch and Shapiro. R. A. Abramovitch and D. Shapiro, J. Chem. Soc., 1954, 4263. R. A. Abramovitch and D. Shapiro, J. Chem. Soc., 1956, 4589.
9. For representative experimental procedures for the synthesis of **8b, 8d** and **8e** see K. Narayanan, L. Schindler, and J. M. Cook, J. Org. Chem., 1991, **56**, 359. K. Narayanan and J. M. Cook, J. Org. Chem., 1991, **56**, 0000.

10. a) E. O. Orlemanes, W. Verboom, M. W. Schelting, D. N. Reinhoudt, P. Lelieveld, H. H. Fiebig, B. R. Winterhalter, J. A. Double, and M. C. Bibby, J. Med. Chem., 1989, **32**, 1612. b) P. Cai and J. K. Snyder, Tetrahedron Lett., 1990, **31**, 969. c) H. Teuber and G. Thaler, Chem. Ber., 1958, **91**, 2253.

11. S. Patai, Ed., 'The Chemistry of the Quinonoid Compounds', Part 1, John Wiley and Sons, New York, 1974.

12. For a recent example see T. Fukuyama, L. Yang, K. L. Ajeck, and R. A. Sachleben, J. Am. Chem. Soc., 1990, **112**, 3712.

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