Acidic Properties of Hypericin and its Octahydroxy Analogue in the Ground and Excited States

Dalia Freeman,[a] Felix Frolow,[a] Evgeny Kapinus,[b] David Lavie,[a] Gad Lavie,[c] Dan Meruelo[c] and Yehuda Mazur*[a]

[a] *Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel*
[b] *Scientific and Engineering Centre, 'Sonar' Vladimirskaya 41, Kiev, Ukraine*
[c] *New York University Medical Center, New York 10016, USA*

pH dependent absorption and fluorescence spectra of hypericin **1** and its octahydroxy analogue **3** demonstrate their acidic properties, their deprotonation occurring in the ground and excited states from OH in the bay region and in the *peri* position to the carbonyl, respectively; the presence of the anion of **1** in the crystalline state is established by X-ray diffraction.

Hypericin **1**, an antiviral agent and potential anti-AIDS drug,[1,2] has been recently found to be photoactive, light being presumably responsible for most of its antiviral activity.[3,4] We show here that **1** is an acid and is present in the pH range between *ca.* 4 and 11 only as a monoanion.

1 obtained by synthesis[5]† was crystallized as needles from pyridine. A single crystal X-ray diffraction study‡ reveals a structure which contains one molecule of **1**, two molecules of pyridine and one of water [Fig. 1(*a*)]. All hydrogen atoms were found from a difference Fourier map and were refined in the free manner. The hypericin molecule is distorted, showing a helical twist, the torsion angles of C(3)–C(3a), C(3b)–C(4) and of C(10)–C(10a), C(10b)–C(11) bonds being 19.2 and 32.4° respectively, [Fig. 1(*b*)]. Relevant intramolecular distances between bonded H atoms and neighbouring O and H atoms are listed in the caption to Fig. 1. The distance between O(3) and O(4) atoms appears to be remarkably short, 2.36 Å, with only one H atom bound to both of them. The missing H is bound to each N atom of both pyridine molecules in a mutually exclusive manner. Refinement of the occupancy factor for this H atom's sites, maintaining equal temperature factor for both, reveals apparently non-symmetric distribution of these sites [0.68(7):0.32(7)]. Thus **1** crystallized from pyridine consists in fact of an ion pair of hypericin anion and pyridinium cation.

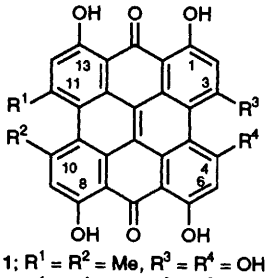
The presence of hypericin anion in the crystalline state suggests that **1** exists also as an acid in organic solvents, the acidic OH being located at the bay region [C(3)/C(4)].

The acidity of **1** was confirmed by pH dependent absorption spectra which reveal three species: the unionized form of **1**, its monoanion and dianion, the respective pK_a values being 1.7 and 12.5.§ The spectrum of the unionized form of **1** was obtained in the absence of acid in dry, nonprotic solvents (*e.g.* THF, EtOAc), while the spectrum due to the monoanion was obtained in Me_2SO, DMF and alcohols (*e.g.* EtOH, MeOH). The latter spectrum was identical to that of Na salts of **1**† formed upon neutralization of **1** with one equiv. NaOH.

The recently described isohypericin **2**,[6] possessing only one OH in each of the bay regions, is a much weaker acid than **1**, its pK_a value derived from pH dependent absorption spectra in 80% EtOH being 7.5, similar to that found in the related emodin, a 7-methyl-1,3,9-trihydroxyanthraquinone (pK_a 6.5). This implies that the facile ionization of **1**, which takes place at OH in the bay region[7] is due to the proximity of the two OH groups in the bay region, one of which is H-bonded to the O atom of the second OH in the sterically crowded environment.

The differences between the ground and excited state acidity, ΔpK_a^*, calculated from both absorption and emission data§ by the Foerster cycle are 0.8 and 3.3 for **1** and its monoanion respectively. The excited state deprotonation occurs from the OH in the vicinity of the carbonyl group, as recently suggested.[8]

The related hypericin analogue, an octahydroxy mesonaphthodianthrone **3** isolated as its disodium salt,[9]¶ which contains two OH groups in each bay region (C3/C4, and C10/C11) shows also acidic properties. pH dependent absorption spectra of **3** reveal four different species: the unionized **3**, its monoanion, dianion and trianion, the respective pK_a values being 1.6, 3.6 and 13.2.¶ The ΔpK_a^* values calculated from the absorption and emission data§ were 0.8, 0.52 and 2.38 for



1; $R^1 = R^2 = Me$, $R^3 = R^4 = OH$
2; $R^1 = R^4 = Me$, $R^2 = R^3 = OH$
3; $R^1 = R^2 = R^3 = R^4 = OH$

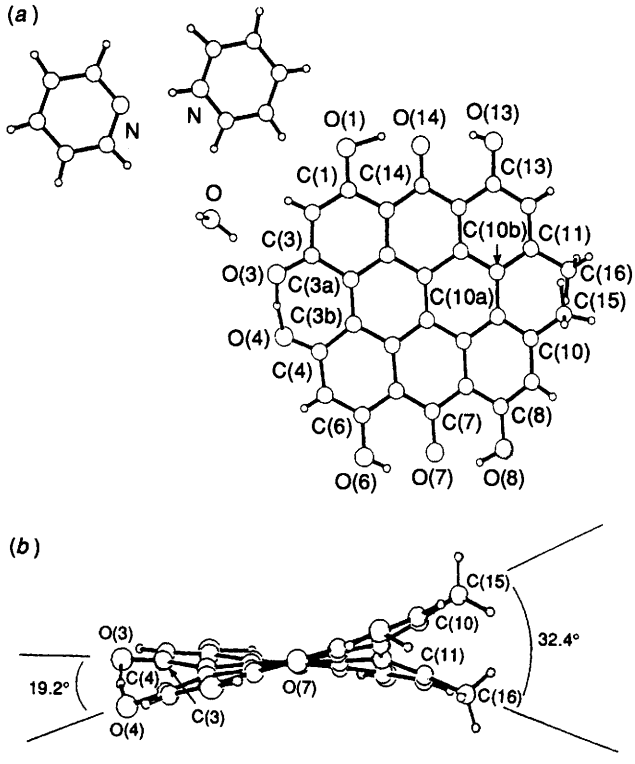


Fig. 1 (*a*) Asymmetric part of the X-ray structure of **1** crystallized from pyridine. Selected distances: O(1)–O(14) 2.497(5), O(13)–O(14) 2.494(4), O(6)–O(7) 2.495(5), O(8)–O(7) 2.529(5), O(3)–O(4) 2.362(4), O(1)–H(1)···O(14) 0.89(6) 1.74(6), O(13)–H(13)···O(14) 1.10(5), 1.52(5), O(6)–H(6)···O(7) 1.13(6) 1.50(6), O(8)–H(8)···O(7) 0.81(6), 1.79(6), O(3)–H(4)···O(4) 1.17(5) 1.20(5). (*b*) View of **1** along O(7)–O(14) axis.

3, its mono- and dianion respectively. Thus the excited state deprotonation of 3 at pH between 4 and 11 can be attributed to its dianion, and occurs also from OH in the peri position to the carbonyl group.

It has been suggested that the photophobic and negative phototactic response of a protozoa, *Stentor coeruleus*, may be initiated by excited state deprotonation of a stentorin-chromophore,[10] a diisopropyl substituted octahydroxy analogue of hypericin.[11] It is reasonable to assume that also in this case its excited state deprotonation occurs from OH in analogous position.

Generous financial support for this work was provided by VIMR$_x$ Pharmaceuticals Inc., Stamford, CT, USA

Received, 4th November 1993; Com. 3/06612F

Footnotes

† *Selected data* for **1**. IR ν/cm^{-1} (KBr): 1625, 1599, 1473, 1369, 1258, 1224, 1188, 1116, 844, 673, 657, 602, 566, 536; ^{1}H NMR (400 MHz, [$^2H_6$]DMSO) δ 14.67 (2H, s, C8, C13-OH), 14.04 (2H, s, C1, C6-OH), 7.35 (2H, s, C2, C5), 6.50 (2H, s, C9, C12), 2.65 (6H, s, CH_3); MS–FAB$^+$: *m/z* 505 (M + H$^+$).

For Na salt of **1**: IR ν_{max}/cm^{-1} (KBr) 1590, 1555, 1501, 1465, 1421, 1259, 1188, 1113, 843, 624, 604, 567. ^{1}H NMR (400 MHz, [$^2H_6$]DMSO) 18.34 (1H, s, C3 or C4-OH) 14.67 (2H, s, C8, C13-OH), 14.06 (2H, s, C1, C6-OH), 7.34 (2H, s, C2, C5), 6.45 (2H, s, C9, C12), 2.66 (6H, s, CH_3); MS–FAB$^+$: *m/z* 504 (M − H + Na$^+$), 527 (M + H$^+$).

For C_5H_5NH salt of **1**:IR ν_{max}/cm^{-1} (KBr) 1586, 1547, 1466, 1426, 1391, 1371, 1344, 1261, 1187, 1146, 1115, 843, 679, 661, 621, 601. ^{1}H NMR (400 MHz, [$^2H_6$]DMSO) 14.65 (2H, s, C8, C13-OH), 14.02 (2H, s, C1, C6-OH), 8.72 (2H, dd, *J* 8, 2 Hz H_α-N) 8.17 (1H, tt, *J* 8, 2, 2 Hz H_γ-N) 7.81 (2H, tt, *J* 8, 2, 2 Hz H_β-N), 7.32 (2H, s, C2, C5), 6.44 (2H, s, C9, C12), 2.65 (6H, s, CH_3).

‡ *Crystal data* for **1** crystallized from pyridine as pyridinium–pyridine–water complex in the form of dark-red needles: ($C_{30}H_{15}O_8 \cdot C_5H_5NH \cdot C_5H_5N \cdot 2H_2O$) M = 679.6, space group $P\bar{1}$ with a = 11.891(6); b = 19.131(4), c = 6.896(2) Å, α = 91.89(3), β = 100.68(2), γ = 107.27(2)°; V = 1465.6(4) Å^3; Z = 2, D_c = 1.54 g cm^{-3}. Data were collected on a Rigaku AFC5R diffractometer mounted on Rigaku RU300 rotating anode generator; Mo-Kα, graphite monochromator (λ = 0.71073 Å); T = 90 K using locally modified Enraf-Nonius type low-temperature device; 6957 reflections collected, 4624 unique reflections with $I > 0$ were utilized in the crystal structure and least-squares refinement to yield R = 0.0615 [calculated with $I > 2\sigma(I)$] and wR = 0.136, based on F; programs used SHELXS-86[12] and SHELXL-92.[13] X-Ray crystal data of a similar structure, however with only one pyridine molecule was recently published. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

§ *Absorption spectra*, λ_{max}/nm (ε); fluorescence spectra, excitation 550 nm [λ_{max}/nm] in 80% EtOH, at selected pH. For **1** at pH 0.6: 580, 535, 460, 320 (40 000, 22 000, 25 000, 33 000) [580]. For $\mathbf{1}^-$ at pH 8.6: 590, 550, 470, 340 (50 000, 24 000, 16 000, 35 000) [602]. For $\mathbf{1}^{2-}$ at pH 13.5: 650, 470, 365 (33 000, 18 000, 41 500) [683]. For **3** at pH 0.6: 574, 535, 440, 345 (33 000, 18 500, 20 000, 35 000) [579]. For $\mathbf{3}^-$ at pH 2.9: 588, 550, 430, 350 (41 500, 21 500, 15 500, 41 000) [592]. For $\mathbf{3}^{2-}$ at pH 8.0: 596, 550, 450, 355 (50 000, 25 000, 17 000, 49 000) [602]. For $\mathbf{3}^{3-}$ at pH 13.5: 622, 450, 365 (28 000, 18 500, 46 500) [665].

¶ *Synthesis* of **3**: reagents and conditions, 1,3,6,8-tetrahydroxy-9.10-anthraquinone (0.5 equiv.) hydroquinone (5 equiv.), KOBu^t (2.5 equiv.) 20 d, 120 °C sealed ampule. Selected data for **3**. IR ν_{max}/cm^{-1}; 1574, 1443, 1418, 1412, 1386, 1277, 1143, 1110; ^{1}H NMR (400 MHz, [$^2H_6$]DMSO) 17.42 (2H, s, C3/C4, C11/C10) 14.94 (4H, s, C1, C6, C8, C13-OH), 7.34 (4H, s, C2, C5, C7, C12).

References

1 G. Lavie, F. Valentine, B. Levin, Y. Mazur, G. Gallo, D. Lavie, D. Weiner and D. Meruelo, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 5963. D. Meruelo, G. Lavie, D. Lavie, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5230. L. Liebes, Y. Mazur, D. Freeman, D. Lavie, G. Lavie, N. Kudler, S. Mendoza, B. Levin, H. Hochster and D. Meruelo, *Anal. Biochem.*, 1990, **195**, 77. D. Lavie, D. Freeman, H. Bock, J. Fleischer, K. van Kranenburg, Y. Ittah, Y. Mazur, G. Lavie, L. Liebes and D. Meruelo, *IUPAC Trends in Medicinal Chemistry 90, Proc. X Int. Symp. Med. Chem.*, Jerusalem, 1992, 321.

2 *cf*: D. O. Anderson, N. D. Weber, S. G. Wood, B. G. Hughes, B. K. Murray and J. A. North, *Antiviral Res.* 1991, **16**, 185. R. F. Schinazi, K. Chu, Ch. J. R. Babu, B. J. Oswald, V. Saalmann, D. L. Cannon, B. F. Eriksson and N. Nasr, *Antiviral Res.* 1990, **13**, 265. J. B. Hudson, I. Lopez-Bazzocchi and G. H. N. Towers, *Antiviral Res.* 1991, **15**, 101.

3 D. Meruelo, S. Degar, N. Amari, Y. Mazur, D. Lavie, B. Levin and G. Lavie, *Natural Products as Antiviral Agents*, ed. C. K. Chu and H. G. Cutler, Plenum NY, 1992, 91. S. Degar, A. M. Prince, D. Pascual, G. Lavie, B. Levin, Y. Mazur, D. Lavie, L. S. Ehrlich, C. Marter and D. Meruelo, *AIDS Research and Human Retroviruses*, 1992, **8**, 1929.

4 S. Carpenter and G. A. Kraus, *Photochem. Photobiol.*, 1991, **53**, 169. J. B. Hudson, L. Harris and G. H. N. Towers, *Antiviral Res.*, 1993, **20**, 178. J. Lenard, A. Rabson and R. Vanderoef, *Proc. Natl. Acad. Sci., USA*, 1993, **90**, 158.

5 Y. Mazur, H. Bock and D. Lavie, Canad. Pat. Appl. 202993, CA **116**, 6343z, 1992.

6 H. Falk and G. Shoppel, *Monatsh. Chem.*, 1992, **123**, 931.

7 H. Falk, J. Meyer and M. Oberreiter, *Monatsh. Chem.*, 1992, **123**, 277. H. Falk, J. Meyer and W. Schmitzberger, *Monatsh. Chem.*, 1992, **123**, 731.

8 F. Gai, M. J. Fehr and J. W. Petrich, *J. Am. Chem. Soc.*, 1993, **115**, 3384.

9 3 is the suggested structure for Fringelite D, a fossile pigment. M. Blumer, *Geochem. Cosmochem. Acta*, 1962, **26**, 225. M. Blumer, *Science*, 1965, **149**, 722.

10 P.-S. Song, D. P. Haeder and K. L. Poff, *Photochem. Photobiol.*, 1980, **32**, 781. D. P. Haeder and M. A. Haeder, *Photochem. Photobiol.*, 1991, **54**, 423.

11 N. Tao, M. Orlando, J.-S. Hyon, M. Gross and P.-S. Song, *J. Am. Chem. Soc.*, 1993, **115**, 2526.

12 G. M. Sheldrick, *Acta Crystallogr Sect A*, 1990, **46**, 467.

13 G. M. Sheldrick, SHELXL-92, Structure refinement program, Institut für Anorganische Chemie der Universität, Göttingen, Germany, 1992.

14 E. Etzlstorfer, H. Falk, N. Mueller, W. Schmitzberger and U. G. Wagner, *Monatsh. Chem.*, 1993, **124**, 751.