smallest calixarene. Here, there would be a barrier to the free rotation of the pyridine ring in the direction that takes the *N*-oxide (**7**) or *N*-hydroxide (**5**, **6**) through the cavity. Compounds **8** and **9** have larger cavities, resulting in lower barrier energies. Compounds **5**, **6**, **8**, and **9** are known to be in the *N*-hydroxide form in solution (see NMR discussion above) while **7** is an *N*-oxide. It is not understood why the conformational barriers for the *N*-hydroxides and *N*-oxide compounds are the same. The tetra-*O*-methyl calix[4]arene, where all the phenolic OH groups were methylated, had about the same barrier to rotation as did the parent calix[4]arene.[27] Likewise in our case, the addition of a hydrogen to the *N*-oxide oxygen for **7** to have **5** or **6** evidently does not cause more steric hindrance for the rotational process.

Compound **6** was found to be an effective transport agent for lithium ions in a water–methylene chloride–water bulk liquid membrane system.[9,10] We have reported that **1** ($n = 0$) is an excellent transporting agent for lithium ions. A comparison of flux values for the transport of lithium ions by **1** ($n = 0$) and **6** is given in Table III. It is apparent that different mechanisms are operative for the transport of lithium ions by **1** ($n = 0$) and **6**. Both crowns exhibited the best transport at high source phase pH values since they need to be ionized before complexation can occur. However, **1** ($n = 0$) gave the best transport into a receiving phase that was neutral, while **6** transported best into an acidic receiving phase. The transport rate for **6** was much lower than that for **1** ($n = 0$). In single-cation systems, crown **6** did not transport to any significanct degree any other cation studied except lithium as mentioned above and silver, which was transported at a flux <1 mol·10^{-6} $s^{-1}\cdot m^{-2}$. Crown **1** ($n = 0$) was selective for lithium ions over all other alkali-metal cations in competitive transport in the bulk liquid membrane system.[10] Compound **6** was likewise a selective transporter of lithium over other alkali-metal cations in competitive cation experiments into a water receiving phase with selectivities of $Li^+/Na^+ = 22$, $Li^+/Rb^+ = 54$, and $Li^+/Cs^+ = 10$. Competitive transport into an acid receiving phase also showed Li^+ selectivity but the deviations were too large to quantify the results.

When crown **1** ($n = 0$) is ionized, the resulting cavity is open and the pyridone ring contains a negative charge. The resulting macroring is able to accommodate a lithium ion in the cavity. Crown **6** would ionize to give a cavity with an *N*-oxide in close proximity which would interfere with an incoming cation so that complexation probably takes place above the ring cavity. A similar complexing mode was observed by Browne and co-workers for an 18-crown-6 containing an intraannular phenolic group.[28] They observed that their carrier transported all the alkali-metal ions in single-cation experiments, possibly because an 18-membered macroring was used. The smaller ring cavity in the case of **6** would be the best size for the lithium ion.

Acknowledgment. Financial support of this work is from the Department of Energy, Office of Basic Energy Sciences, Grant No. DE-FG02-86ER13463. We thank Mr. Bryon J. Tarbet for help in obtaining the temperature-dependent NMR spectra.

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Electrochemical Oxidation of 5-Hydroxytryptamine in Acidic Acetonitrile

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The electrochemical oxidation of 5-hydroxytryptamine (AH) in acidic acetonitrile yields predominantly (80%) a 3,4′ dimer (indoleninium, indole) whose structure has been established by means of ^1H and ^{13}C NMR and other analytical techniques. On the basis of cyclic voltammetry a mechanism is proposed. The initial heterogeneous one-electron abstraction (standard redox potential $E°$) produces a C(3)• radical cation $AH^{•+}$ and is followed by a 3,4′ radical–substrate coupling yielding a dimer radical cation $DH^{•+}$. A disproportionation-like second-electron transfer occurring in solution between $AH^{•+}$ and $DH^{•+}$ gives AH (half-regeneration) and DH_2^{2+}, which deprotonates at position 4′, that deprotonation being the rate-determining step of the whole process. An estimation of $E°$ is possible: $+925 < E° < +950$ mV vs SCE.

The biological interest of studying the oxidation of 5-hydroxytryptamine (5HT) has been summarized in a recent paper.[2] The authors described the products formed at micromolar concentrations through electrochemical oxidation controlled to a significant extent by the adsorption of reactant and product(s), and they also proposed a mechanism for the electrochemical process. Their experiments were carried out in acidic (pH ~2) aqueous media using pyrolytic graphite electrodes.

The present paper deals with the electrochemical oxidation of 5HT under conditions that permit the obtention of signals free of adsorption interference and allow mechanistic deductions through careful cyclic voltammetric analysis. The results we report show that use of a nonaqueous solvent such as acetonitrile (ACN) and platinum electrodes prevents the occurrences of appreciable adsorption and electrode fouling, even when the 5HT concentration lies in the millimolar range. Addition of 1 M $HClO_4$ is motivated by two reasons: The final products are probably less prone to spontaneous oxidation in such

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an acidic medium, and the deprotonations of the intermediates are surely slower than in less acidic solutions. The latter consideration may be of mechanistic importance if the initial electron transfer is followed or accompanied by proton abstraction as said in the reaction pathway given in ref 2.

Our cyclic voltammetric results enable us to propose a new mechanism for the formation of only one dimeric major product, along with minor products thought to be due to its chemical transformation. This dimer is apparently identical with one of the products isolated after electrochemical oxidation in aqueous acidic media. However, the structure that was then formulated as 1,4′-bi-5-hydroxytryptamine[2] seems erroneous and we propose a new one.

Cyclic Voltammetry

In ACN (1.05 M $HClO_4$), when the potential is swept from E_i = +680 mV vs SCE[3] to E_f = +980 mV and backward, the signal due to the presence of 5HT in solution (0.96 mM) consists of a single well-defined anodic peak P_a whose peak potential is E_{pa} = +855 mV when the potential sweep rate v is 0.2 V s^{-1}. No cathodic peak can be observed on the reverse sweep even at v as high as 200 V s^{-1}; therefore P_a results from the irreversible oxidation of 5HT. The P_a peak characteristics are as follows: $i_{pa}/v^{1/2}C$ does not depend on v and C (i_{pa} being the peak height and C the 5HT bulk concentration) (i.e., there is no adsorption of either the reactant or the product(s) at the ACN/Pt interface); the $i_{pa}/v^{1/2}C$ ratio corresponds to a one-electron transfer; $E_{pa} - E_{pa/2}$ = 38.0 ± 1.5 mV, whatever v in the range 0.1–50 V s^{-1} ($E_{pa/2}$ being the potential at mid peak height), $\partial E_{pa}/\partial \log v$ = 20 ± 1 mV and $\partial E_{pa}/\partial \log C$ = 19 ± 2 mV (0.4 < C < 4.0 mM).

Our study was restricted to the 0.2–1.1 M $HClO_4$ concentration range. The lower limit is a threshold for a correct separation between P_a and a second anodic signal that appears in this potential range at low $HClO_4$ concentration. The upper limit corresponds to the transformation of <10% of 5HT into its acidic form, which absorbs at λ_{max} (ϵ_{max}) 248 nm (4700 M^{-1} cm^{-1}) and 322 nm (4700 M^{-1} cm^{-1}). As the pK_a of $HClO_4$ in ACN is given in the literature (pK_a(ACN) = −8.8),[4] it is possible to determine the pK_a for the protonation of the indole ring of 5HT under those conditions. We found pK_a(ACN) = −1.0 ± 0.1. In the narrow range of $HClO_4$ concentration used in this work, E_{pa} does not shift with increasing $HClO_4$ concentration at given v and C.

Controlled-Potential Electrolysis

Electrolysis was stopped when ca. 95% of 5HT had been consumed. Under these conditions the experiment gave a faradaic n value of 1.1 ± 0.1. Then a major dimeric product was isolated (see Experimental Section) and will be termed D further in the text.

The spectrum of the electrolyzed acidic solution exhibits UV absorption signals at positions λ_{max} 283 and 300 nm (sh) (Figure 1, curve b), which are those expected for DH^+. However, careful analysis of this spectrum also indicates the presence of minor components that absorb in the region 380–480 nm and are responsible for an absorbance increase at ca. 305 nm. After addition of aqueous $KHCO_3$ as described in the Experimental Section, the UV–visible spectrum shows only a double absorption band at 282 nm and 310 nm (Figure 1) characteristic of D.

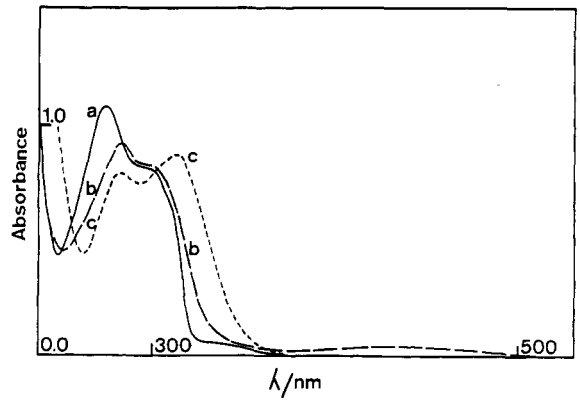
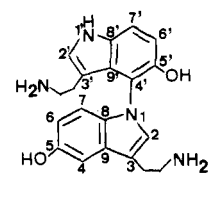


Figure 1. UV–visible spectrometry: curve a (—), 2 mM 5HT before electrolysis at +840 mV; curve b (– – –), after electrolysis; curve c (- - -), after addition of aqueous $KHCO_3$ to the latter solution until pH ~4 (corrected for dilution).

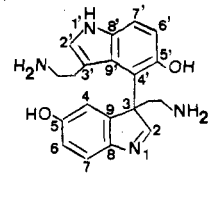
A HPLC chromatogram of the electrolyzed solution clearly exhibits one major peak characterized by an area ratio ((area at 280 nm)/(area at 310 nm)) and a retention time identical with those of pure D. DH^+ is found to be produced in 80 ± 5% yield through HPLC assay (see Experimental Section). The complexity of the minor products mixture formed in addition to DH^+ is evidenced from observation of several not well-resolved HPLC peaks corresponding to compounds that are more rapidly eluted than D. However, the profile of products (except 5HT) eluted in this region of the chromatogram is quite similar to the one that arises during the transformation of pure D in acidic ACN.

Structure of D

FAB-MS along with elemental analyses establishes that D is a one-electron oxidation dimer of 5HT. UV–visible and ^1H NMR data obtained for D are completely comparable to those reported recently for a dimer that resulted from oxidation of 5HT in aqueous media[2] and that was identified as 1,4′-bi-5-hydroxytryptamine (structure I) on the basis of the most notable features of its ^1H NMR spectrum (DMSO-d_6): (i) Only one indolic nitrogen proton N(1′)–H (δ 11.22) is observed; (ii) the signal from the proton adjacent to the nitrogen N(1) appears as a singlet (δ 6.10); and (iii) the proton at position C(4′) is missing and correspondingly long-range coupling for C(6′)–H has been lost. However, these data allow only the assignment of one of the positions of the linkage between the two "indole" moieties as being at C(4′), the second position being either at N(1) as in structure I or at C(3) as in structure II.



I



II

Examination of the high-field region of the ^1H NMR spectrum of D in D_2O shows an unresolved multiplet centered at δ 3.29 corresponding to the ethylamino side chain of a 5HT moiety and a complex system for the other chain at δ 2.86 (m, 1 H), 2.65 (m, 2 H), and 2.43 (m, 1 H) that could be consistent with the presence of the chiral center at position 3 in structure II: The two methylene protons adjacent to the indolenine ring are not chemical shift equivalent; as a result of the shielding it experiences

(3) All potentials in this work are referred to the KCl saturated calomel electrode (SCE) at 15 °C.

(4) Barrette, W. C.; Johnson, H. W.; Sawyer, D. T. *Anal. Chem.* **1984**, *56*, 1890–1902.

by the quaternary carbon C(3), one of these protons resonates at much higher field (δ 2.43 in D_2O and δ 2.38 in DMSO-d_6) than the two methylene protons adjacent to the indole ring in 5HT ($\delta \sim 3.0$ in both solvents). Comparison of the ^{13}C NMR spectrum of D to that of 5HT coupled with ^{13}C DEPT experiments reveals significant differences that confirm the assignment to D of structure II. In particular, the ^{13}C NMR spectrum (D_2O) clearly exhibits only one signal typical of the carbon at position 3 in 5HT (δ 109.7 in D and δ 110.7 in 5HT) with a new signal at δ 62.7, assignable to a quaternary carbon, which must be the carbon at position 3 in structure II. It is noteworthy that the C(2)–H proton of D (δ 6.25 in D_2O) is markedly shielded relative to its resonance position in 3,3-disubstituted indolenines, which commonly occurs near δ 8.0.[5] While inspection of Dreiding models does not indicate any particular shielding effect by the 5HT moiety, it seems that the presence of the hydroxyl group at position C(5) could cause an extensive polarization of the π-electron cloud with the result of an increase of the net π charge at position C(2) as occurs at this position in 5HT when compared to tryptamine.[6]

Concerning DH^+, its UV–visible spectrum is quite similar to that of 5HT. This observation indicates that DH^+ is an indoleninium species that can undergo transformation when generated in sufficiently strong acidic media.[5]

Mechanism of the Electrochemical Oxidation of 5HT in Nonaqueous Acidic Media

The preceding results show that the oxidative electrodimerization of 5HT is actually a dehydrodimerization since the overall process can be written as shown in eq 1,

$$2AH \rightarrow DH^+ + 2e^- + H^+ \qquad (1)$$

where AH symbolizes the molecular form under which 5HT predominates in these acidic media ACN, and DH^+ represents the dimeric species minus one proton.

The formal kinetics for voltammetric studies of reductive electrohydrodimerizations have been treated thoroughly in the literature.[7] Although not straightforward, the transposition to oxidative electrodehydrodimerizations can be performed easily (see the examples given in the Experimental Section).

The data of cyclic voltammetry ($E_{pa} - E_{pa/2}$; $\partial E_{pa}/\partial \log v$; $\partial E_{pa}/\partial \log C$; totally irreversible one-electron process) fit the formal characteristics of a limited set of four possible mechanisms.[7] Three of these mechanisms involve a radical–radical coupling (rrc) while the last one involves a radical–substrate coupling (rsc).

The production of the radical species results from the heterogeneous one-electron abstraction that occurs at the solution/electrode interface (eq 2, standard redox potential $E°$).

$$AH \overset{E°}{\rightleftharpoons} AH^{\bullet+} + e^- \qquad (2)$$

This electron transfer is assumed to be Nernstian. The consecutive chemical reactions are also assumed to be so fast that a stationary state is established by mutual compensation of the diffusion and the rate-determining chemical steps (rds) and also between the chemical reactions that produce and destroy the intermediary species so that "pure kinetic" conditions can be considered to prevail.[7] That all these assumptions are correct is ascertained by the fact that $E_{pa} - E_{pa/2}$ does not depend on v.[7]

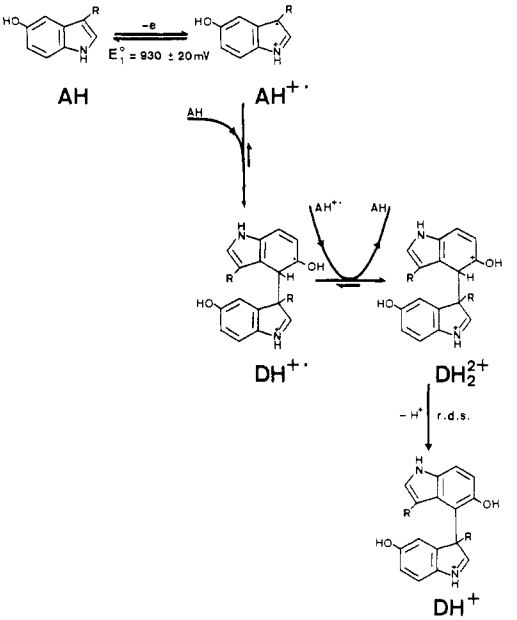


Figure 2. Mechanism of the electrochemical oxidation of 5HT in acidic medium. R = $CH_2CH_2NH_3^+$.

The first two formally possible rrc imply a dimerization of $AH^{\bullet+}$ followed by deprotonation of the dimer and must be disregarded since obtaining a dissymmetrical dimer as the major product through the dimerization of two identical $AH^{\bullet+}$ radicals is very unlikely.

The third formally possible rrc involves the coupling between $AH^{\bullet+}$ and its conjugate base A^-, this coupling reaction being the rds.[7] Then formal kinetics (see Experimental Section) show that a tenfold increase in C_{H^+} should provoke a 19-mV positive shift of E_{pa}. As E_{pa} remains practically unchanged when the $HClO_4$ concentration is increased from 0.2 to 1.0 M, this rrc mechanism must not be retained.

The rsc between the radical cation and the parent compound followed by solution electron transfer and deprotonation of the dimer, this last step being the rds, is the only possible mechanism left, and it is also the only one that rationalizes satisfactorily all the cyclic voltammetric results. This mechanism consists of the reactions given in eq 2–5:

$$AH^{\bullet+} + AH \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} DH_2^{\bullet+} \qquad K_3 = k_3/k_{-3} \qquad (3)$$

$$DH_2^{\bullet+} + AH^{\bullet+} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} DH_2^{2+} + AH \qquad K_4 = k_4/k_{-4} \qquad (4)$$

$$DH_2^{2+} \xrightarrow{k_5} DH^+ + H^+ \qquad (5)$$

That no reversibility (even partial) can be observed on the cyclic voltammogram even at v as high as 200 V s^{-1} shows that the apparent dimerization rate constant ($k_{app} = 2K_3K_4k_5$) is $>10^8$ M^{-1} s^{-1}.[8,9] On the other hand, k_{app} cannot overcome the diffusion limit of ca. 10^{10} M^{-1} s^{-1}. Then an estimation of $E°$ can be performed. We found $+925 < E° < +950$ mV since $E_{pa} = +855 \pm 3$ mV at $v = 0.2$ V s^{-1} and $C = 0.96$ mM in ACN, 1.05 M $HClO_4$.

The cyclic voltammetric behavior of 5MT is totally similar to that of 5HT, a result that indicates that the

(5) Jackson, A. H.; Smith, P. *Tetrahedron* **1968**, *24*, 2227–2239.
(6) Biagini, S.; Lai, A.; Monduzzi, M.; Saba, G. *J. Chem. Soc., Faraday Trans. 2* **1983**, *79*, 491–496.
(7) Nadjo, L.; Savéant, J. M. *J. Electroanal. Chem.* **1973**, *44*, 327–366.
(8) Andrieux, C. P.; Nadjo, L.; Savéant, J. M. *J. Electroanal. Chem.* **1970**, *26*, 147–186.
(9) Andrieux, C. P.; Nadjo, L.; Savéant, J. M. *J. Electroanal. Chem.* **1973**, *42*, 223–242.

deprotonation of the hydroxy group does not play a significant role in the mechanism, opposite to what has been suggested in ref 2. In the case of 5MT, it appears that $E°$ is more positive than in the case of 5HT by an amount of 52 ± 3 mV.

Conclusion

The experiments carried out in the present work show that the electrochemical oxidation of 5HT in nonaqueous acidic media proceeds according to the reaction scheme in Figure 2. We would like to emphasize a few points by comparison with recently published results concerning the electrochemical oxidation of 5HT in acidic aqueous media.[2]

(1) The dissymmetrical dimer obtained in aqueous media and the one isolated in this work exhibit similar chromatographic behaviors and the same ^1H NMR spectra. Therefore we are almost undoubtedly dealing with the same product. The use of ^{13}C NMR as an additional technique enabled us to establish that this dimer results from a 3,4′ coupling instead of the 1,4′ coupling reported in ref 2.

(2) HPLC measurements show that the electrochemical oxidation of 5HT at millimolar concentrations in acidic ACN yields the 3,4′ dimer as the major product, whereas the dissymmetrical dimer is said to be only a minor product at micromolar concentrations in acidic aqueous media. Under those latter circumstances, it is surely difficult to isolate relatively large quantities of that product, and maybe that is the reason why no ^{13}C NMR study was performed. However, a close look at the chromatogram reproduced in Figure 3 of ref 2 reveals that the area under the peak ascribed to this dissymmetrical dimer is not markedly smaller than the area under the peak ascribed to the 4,4′ dimer which is counted among the three major products. Since the molar absorbances of the two dimers are probably not very different at the detection wavelength of 260 nm, the dissymmetrical dimer may not be that minor a product in aqueous acidic media.

Experimental Section

Materials. 5-Hydroxytryptamine creatinine sulfate complex (5HT), 5-hydroxytryptamine hydrochloride, and 5-methoxytryptamine (5MT) were obtained from Sigma. Acetonitrile (ACN) (Spectrosol purity grade) and deuteriated solvents were obtained from SDS. Trifluoroacetic acid (TFA) (for sequential analysis) was from Fluka. Perchloric acid (70%), methanol (HPLC grade), and other reagent-grade chemicals were obtained from Prolabo Chemicals. All the chemicals were used as received.

Elemental analyses were performed at the CNRS Center of Microanalysis (Gif-sur-Yvette).

Electrochemistry. Electrochemical studies were performed in ACN with 1.05 M $HClO_4$ as supporting electrolyte unless otherwise specified. Apparatus was as described previously.[10]

For cyclic voltammetry, the working electrode was a polished platinum disk (surface area of 0.78 mm^2) sealed in soft glass. For controlled-potential electrolysis, two coaxial cylindrical platinum grids were used as the working electrode (total geometrical area ca. 140 cm^2, 96 meshes/cm^2).

Spectroscopic Methods. UV–visible absorption spectra were recorded on a Beckman Acta Century III spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained with a Bruker AM 300 WB apparatus. Fast atom bombardment (FAB) was performed at the CNRS Center for Mass Spectrometry (Lyon).

Chromatography. High-performance liquid chromatography (HPLC) analyses were carried out with a Waters Associates system consisting of a Model 510 solvent delivery system, a Model U6K manual injector (fitted with a 20-μL loop), and a Model 440 UV–visible detector (with wavelength filters fixed at 280 or 313 nm). Separations were performed isocratically at room temperature on a Waters Nova-Pak C_{18} stainless steel column (3.9-mm i.d. × 15 cm), 4-μm particle size) preceded by a Waters Guard-Pak C_{18} guard column. The mobile phase was H_2O/MeOH (90/10) (v/v) containing 3 mM triethylamine (TEA) adjusted to pH 3.75 with TFA. A flow rate of 1.0 mL min^{-1} was used.

Typically the progress of the electrolysis of 5HT in ACN (1.05 M $HClO_4$) was followed by removing 80-μL aliquots at various time intervals and diluting them with 2 mL of the HPLC eluent containing 5MT as an internal standard (concentration of 5MT was chosen as half of the concentration of the starting compound). Aliquots (10 μL) of the mixtures were injected into the HPLC system. Quantitations of the major product formed upon electrolysis, denoted D (HPLC retention time, t_R = 9.3 min), and unreacted 5HT (t_R = 4.9 min) were based on peak area measurements at 280 nm referred to that of 5MT (t_R = 22.5 min) and corrected for variances in the molar absorbances among the different compounds.

Preparative separations were performed by column chromatography using a 2-cm-i.d. glass column packed with 55-cm Sephadex LH 20 (Pharmacia) and a UV detector set at 310 nm. Two eluents were used: (I) H_2O/MeOH (85/15) (v/v) adjusted to pH 2.5 with HCl and (II) H_2O adjusted to pH 2.7 with HCl, at flow rates of 40 and 24 mL h^{-1}, respectively.

Product D: Isolation Procedure. Controlled-potential electrolysis was usually carried out at +840 mV, under nitrogen bubbling, with 180 mL of ACN (1.05 M $HClO_4$) containing 146 mg of 5HT (including creatinine sulfate). Immediately after the end of the electrolysis, the brownish-yellow solution was diluted with 100 mL of H_2O. The pH of the mixture was then increased to ca. 4 by adding cautiously a saturated aqueous $KHCO_3$ solution (additional peaks were observed in HPLC chromatograms when the reaction mixture was neutralized to pH ca. 7). The $KClO_4$ that precipitated was filtered and the almost colorless filtrate was concentrated in vacuo (on a rotary evaporator) to a final volume of ca. 20 mL. The mixture was then filtered; the filter cake was washed several times with EtOH, and the combined filtrates were concentrated in vacuo. The latter step was repeated until the last evaporation left a liquid residue free of solid particles. This residue was then diluted with an equal volume of eluent I, filtered, and subjected to column chromatography using eluent I. The chromatogram showed only one major peak (retention volume of ca. 480 mL) corresponding to product D. This product was purified by column chromatography using eluent II, affording D as a greenish-yellow solid (mp >260 °C) whose purity was assayed by HPLC.

3-(2-Aminoethyl)-3-[3′-(2-aminoethyl)-5′-hydroxyindol-4′-yl]-5-hydroxyindolenine (D): Identification. ^1H NMR (300 MHz, D_2O) δ 2.43, 2.65, 2.86 (m, m, m, 1 H, 2 H, 1 H, C(3)-CH_2CH_2), 3.29 (m, 4 H, C(3′)CH_2CH_2), 6.25 (s, 1 H, C(2)–H), 6.71 (dd, 1 H, $J_{6,7}$ = 9 Hz, $J_{6,4}$ = 3 Hz, C(6)–H), 6.75 (d, 1 H, $J_{6',7'}$ = 9 Hz, C(6′)–H; irradiation at δ 7.23 gives a singlet), 6.88 (d, 1 H, $J_{7,6}$ = 9 Hz, C(7)–H), 7.04 (d, 1 H, $J_{4,6}$ = 3 Hz, C(4)–H), 7.18 (s, 1 H, C(2′)–H), 7.23 (d, 1 H, $J_{7',6'}$ = 9 Hz, C(7′)–H); ^1H NMR (300 MHz, DMSO-d_6) δ 2.38, 2.64–2.74 (m, m, 1 H, 3 H, C(3)CH_2CH_2), 3.35–3.43 (m, 4 H, C(3′)CH_2CH_2), 5.93 (br s, 2 H, 2 × OH), 6.10 (s, 1 H, C(2)–H), 6.48 (m, 2 H, C(6)–H and C(6′)–H), 6.62 (d, 1 H, $J_{7,6}$ = 9 Hz, C(7)–H), 6.71 (d, 1 H, $J_{4,6}$ = 3 Hz, C(4)–H), 7.19 (d, 1 H, $J_{7',6'}$ = 9 Hz, C(7′)–H), 7.38 (d, 1 H, $J_{2',1'}$ = 2.5 Hz, C(2′)–H), 8.01 (br s, 3 H, NH_3^+), 8.22 (br s, 3 H, NH_3^+), 11.22 (d, 1 H, $J_{1',2'}$ = 2.5 Hz, N(1′)–H); ^{13}C NMR (75 MHz, D_2O) δ 28.6, 36.4, 38.0, 41.6 (2 × $CH_2CH_2NH_3^+$), 62.7 (C(3)), 104.2 (C(4)), 108.6 (C(6′)), 109.7 (C(3′)), 115.0 (C(7)), 116.2 (C(6)), 117.3 (C(4′)), 118.6, 118.9 (C(7′) and C(2)), 124.1 (C(9)), 127.0 (C(2′)), 135.8, 136.7, 138.2 (C(8), C(8′), and C(9′)), 154.4, 156.1 (C(5) and C(5′)); FAB-MS (thioglycerol matrix, positive ion) m/z (relative abundance) 453 ($MH_2ClO_4^+$, 7.3), 452 (5.5), 451 ($MH_2ClO_4^+$, 18.5), 351 (MH^+, 100), 350 (M, 27.5), 334 (M – H_2O, 17.1). Anal. Calcd for $C_{20}H_{22}N_4O_2 \cdot 2HClO_4 \cdot HCl \cdot 2H_2O$: C, 38.5; H, 4.65; N, 8.98; O, 30.8; Cl, 17.1. Found: C, 38.6; H, 4.68; N, 8.64; O, 30.5; Cl, 17.4. D is isolated as a mixed perchlorate/chloride (2/1) salt; i.e., the amino side chains and the indolenine ring are protonated. UV–visible, λ_{max} (nm) (ϵ_{max} (M^{-1} cm^{-1})), in H_2O (D is insoluble in neutral ACN), 310 (8100), 282 (7450); in ACN (1.05 M $HClO_4$), 300 (sh), (7400), 283 (8800).

(10) Anne, A.; Moiroux, J. *J. Electroanal. Chem.* **1982**, *137*, 293–306.

That the controlled-potential electrolysis produced the monoprotonated form DH^+ is evidenced by the two following results: (i) UV–visible spectrometry in aqueous media indicates that the first protonation of D occurs with a pK_a of ca. 0.6 to give DH^+. On the basis of these spectral changes the spectrum of D in ACN (1.05 M $HClO_4$) corresponds to that of the only acidic species DH^+; (ii) HPLC analysis of a solution of DH^+ prepared in ACN (1.05 M $HClO_4$) and chromatographed under the same conditions as those used for the electrolyzed solution of 5HT reveals the presence of only one HPLC peak due to D; i.e., the buffer capacity of the HPLC eluent is efficient enough to convert remaining DH^+ to D.

Formal Kinetics. The rsc mechanism consists of the reactions given in eq 2–5 (see text). In the present case, reaction 5 is practically irreversible since DH^+ is the only major product that exists at equilibrium after electrolysis. The stationary-state approximation is assumed concerning $DH_2^{\bullet+}$ and DH_2^{2+}. Deprotonation (eq 5) is the rds if $k_5 \ll k_{-4}C_{AH}$ and at the same time $k_{-3} \gg K_4k_5C_{AH^{\bullet+}}/C_{AH}$. It follows that the apparent rate constant is $k_{app} = 2K_3K_4k_5$; then $E_{pa} = E^\circ + 50.6 - 19 \log K_3K_4k_5Cv^{-1}$ and $E_{pa} - E_{pa/2} = 37.5$ mV, the potentials being expressed in mV and the temperature being 15 °C.

The rrc mechanism would consist of the reactions given in eq 2 (see text), 6, and 7:

$$AH^{\bullet+} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} A^\bullet + H^+ \qquad K_6 = k_6/k_{-6} \tag{6}$$

$$A^\bullet + AH^{\bullet+} \xrightarrow{k_7} DH^+ \tag{7}$$

The stationary-state approximation can be performed by treating $A^\bullet$ as a reactive intermediate. Coupling 7 is the rate-determining step (rds) as soon as $k_{-6}C_{H^+} \gg k_7C_{AH^{\bullet+}}$. Now the apparent rate constant becomes $2K_6k_7/C_{H^+}$; then $E_{pa} = E^\circ + 50.6 - 19 \log K_6k_7C/C_{H^+}v$ and $E_{pa} - E_{pa/2} = 37.5$ mV.

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Supplementary Material Available: One figure giving the HPLC chromatogram of the reaction mixture resulting from the electrolysis of 5HT in acidic ACN, and the mathematical treatments of the formal kinetics (4 pages). Ordering information is given on any current masthead page.

Structure of FR900359,[1] a Cyclic Depsipeptide from *Ardisia crenata sims*

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The structure of FR900359, a novel cyclic depsipeptide from *Ardisia crenata sims* that shows inhibition of platelet aggregation and decrease of blood pressure, has been determined as 3-acetamido-22-benzyl-10-[1-[(3-hydroxy-4-methyl-2-propionamidopentanoyl)oxy]-2-methylpropyl]-4-isopropyl-7-(1-methoxyethyl)-19-methylene-8,13,14,16,20-pentamethyl-1,5-dioxa-8,11,14,17,20-pentaazacyclodocosane-2,6,9,12,15,18,21-heptone by hydrolytic, ^1H and ^{13}C nuclear magnetic resonance spectroscopic, and mass spectrometric studies. FR900359 consists of ten units: alanine, *N*-methylalanine, β-hydroxyleucine (three residues), 3-phenyllactic acid, acetic acid, propionic acid, and the uncommon amino acids *N*-methyldehydroalanine and *N,O*-dimethylthreonine.

Introduction

Ardisia crenata sims is an evergreen plant that grows abundantly in the Far East and is widely used as an ornamental plant in Japan. As a result of the continuing search for potentially therapeutic materials in natural products, we found that a cyclic depsipeptide, code-named FR900359, isolated from a methanol extract of the whole plants of *Ardisia crenata sims*, inhibits platelet aggregation in rabbits in vitro, decreases the blood pressure, and causes dose-related hypotension in anesthetized normotensive rats.[3] It is cytotoxic to cultured rat fibroblasts and myelocytic leukemia cells.

We report the structure determination of FR900359.[4] Of particular significance of this cyclic depsipeptide is the uncommon amino acid *N*-methyldehydroalanine found previously only in a toxin from the blue-green alga *Mi-*

Table I. ^1H and ^{13}C NMR Spectral Data of FR900359

^1H chemical shift,[a,b] δ	nature of hydrogens
0.8–1.4 (m,[c] ca. 30)	aliphatic methyl
1.9–5.4 (m,[c] ca. 20)	methylene, methine
2.21 (s, 3), 2.67 (s, 3), 2.87 (s, 3), 3.14 (s, 3), 3.39 (s, 3)	*O*-methyl, *N*-methyl, acetyl
6.74 (d, 1), 6.84 (d, 1), 7.12 (d, 1), 7.56 (d, 1), 8.50 (d, 1)	amide CH–NH–CO, alcohol CH–OH
7.28 (s, 5)	aromatic hydrogen
^{13}C chemical shift,[d] δ	**nature of carbons[e]**
10–73 (ca. 30)	methyl, methylene, methine
106.7 (1)	olefinic $=CH_2$
126.9 (1), 128.5 (2), 129.6 (2)	aromatic =CH
136.0 (1), 145.3 (1)	aromatic =C, olefinic =C
163.8 (1), 166.4 (1), 167.7 (1), 169.2 (1), 169.9 (1), 170.2 (1), 171.2 (1), 171.3 (1), 172.4 (1), 174.7 (1)	amide CO, ester CO

[a] Multiplicity: s; singlet, d; doublet, m; multiplet. [b] Number of hydrogens. [c] Assignments of these signals were difficult, because of their insufficient splitting. [d] Number of carbons. [e] Nature of carbons was proposed by the chemical shift values and proton off-resonance experiments.

(1) FR900359 for this cyclic depsipeptide is a code number used by Fujisawa Pharmaceutical Co., Ltd.

(2) Most of this work has been carried out at the Department of Chemistry, Massachusetts Institute of Technology, during a sabbatical leave (M.F.) from Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.

(3) Horiai, H.; Shimizu, I.; Shibayama, F.; Kikuchi, H., unpublished results.

(4) A preliminary investigation of the structure of FR900359 had been carried out in collaboration with Masataka Shigi, Keiichi Nakashima, Isamu Sumino, and Sueo Atarashi at Analytical Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.

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