in a polyethylene bottle open to the air, was added 0.65 mL (15 mmol) of concentrated hydrofluoric acid. The solution was stirred for 10 h, at which time 1.35 mL (19 mmol) of concentrated ammonium hydroxide was added. The solution was poured into a flask and the acetonitrile removed in vacuo. The resulting suspension was washed with water and extracted with dichloromethane and the extract dried (Na_2SO_4/K_2CO_3) and concentrated under reduced pressure. Chromatography of the residue eluting with 30% ethyl acetate in hexanes, followed by daily elutions of the column for the next 3 days, afforded 12.7 mg (85%) of spiroketal **34**: IR ($CHCl_3$) 1780, 1715 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.84 (d, 3 H, J = 7.0 Hz, *23*), 0.91 (d, 3 H, J = 7.0 Hz, *19*), 0.96 (d, 3 H, J = 6.9 Hz, *20*), 1.09 (d, 3 H, J = 7.1 Hz, *24*), 1.14 (s, 3 H, *14*), 1.21 (d, 3 H, J = 7.0 Hz, *21*), 1.30 (s, 3 H, *13*), 1.8–2.15 (m, 4 H, *10* and *11*), 2.16 (qt, 1 H, J = 8.7, 2.4 Hz, *4*), 2.24 (dd, 1 H, J = 14.5, 1.0 Hz, *8* equat), 2.34 (quint d, 1 H, J = 7.0, 3.9 Hz, *18*), 2.57 (qdd, 1 H, J = 7.2, 3.1, 1.0 Hz, *6*), 2.79 (d, 1 H, J = 14.5 Hz, *8* axial), 3.36 (s, 3 H, O*Me*), 3.45 (dd, 1 H, J = 6.6, 4.9 Hz, *3*), 3.76 (dd, 1 H, J = 9.1, 3.1 Hz, *5*), 4.05 (dq, 1 H, J = 8.3, 6.8 Hz, *2*), 4.16 (dd, 1 H, J = 8.9, 2.6 Hz, *16*), 4.23 (t, 1 H, J = 8.3 Hz, *16*), 4.36 (ddd, 1 H, J = 7.7, 3.9, 2.6 Hz, *15*); ^{13}C NMR ($CDCl_3$) δ 10.5, 11.5, 13.9, 14.9, 17.9, 28.6, 28.7, 29.3, 37.0, 37.1, 38.7, 38.8, 46.6, 48.0, 58.9, 63.5, 65.3, 71.7, 81.4, 84.1, 107.3, 126.9, 127.5, 128.5, 141.1, 153.4, 176.0, 210.3; mass spectrum (CI^-) M - 1 peak calcd for $C_{24}H_{38}NO_7$ 452.2649, found 452.2654.

(4*S*)-3-[(2*S*,3*R*,4*S*)-3-Methoxy-2-methyl-4-[(2*R*,3*R*,4*S*,6*R*)-4-hydroxy-3,8,8-trimethyl-1,7-dioxaspiro[4.5]dec-2-yl]pentanoyl]-4-isopropyloxazolidin-2-one (35). To a stirred solution of 35.3 mg (78 μmol) of ketone **34** in 1 mL of THF cooled to -78 °C with a Flexi-cool cooling bath was added 93 μL (93 μmol) of a 1 M K-Selectride solution. The resulting reaction mixture was stirred for 22 h, at which time 1.5 mL of a pH 7 phosphate buffer was added. The reaction mixture was extracted with dichloromethane and the extract dried (Na_2SO_4/K_2CO_3) and concentrated under reduced pressure. Chromatography of the residue eluting with 25% ethyl acetate in hexanes gave 21.6 mg of spiroketal **35** (61%) and 8.6 mg of recovered ketone **34**, affording an 81% yield of compound **35** based on recovered starting material. Crystallization of this material from hexane gave sharp-melting (mp 124–125 °C) crystals suitable for X-ray analysis. Spiroketal **35** had the following properties: IR ($CHCl_3$) 3550–3300 (br, OH), 1778, 1695 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.84 (d, 3 H, J = 7.1 Hz, *24*), 0.85 (d, 3 H, J = 6.9 Hz, *20*), 0.89 (d, 3 H, J = 7.0 Hz, *19*), 0.96 (d, 3 H, J = 6.7 Hz, *23*), 1.16 (s, 3 H, *14*), 1.22 (d, 3 H, J = 7.0 Hz, *21*), 1.34 (s, 3 H, *13*), 1.55 (ddd, 1 H, J = 19.8, 5.9, 0.5 Hz, *8* equat), 1.6–2.1 (m, 7 H), 2.33 (quint d, 1 H, J = 7.0, 4.0 Hz, *18*), 3.37 (s, 3 H, *Me*O), 3.54 (dd, 1 H, J = 6.71, 3.84 Hz, *3*), 3.67 (m, 1 H, *7*), 3.72 (dd, 1 H, J = 10.5, 2.2 Hz, *5*) 3.97 (d, 1 H, J = 9.1 Hz, O*H*), 4.20 (m, 3 H, *2* and *16*), 4.52 (m, 1 H, *15*); ^{13}C NMR ($CDCl_3$) δ 10.8, 11.1, 14.9, 15.0, 17.9, 28.6, 28.8, 30.0, 35.5, 35.6, 36.2, 37.5, 38.0, 39.0, 58.4, 58.7, 63.5, 67.6, 71.5, 80.4, 84.4, 107.0, 153.5, 176.6; mass spectrum, m/e (relative intensity) (FAB^+) 454 (46, M - 1), 438 (75), 228 (100). Anal. Calcd for $C_{24}H_{40}NO_7$: 454.2805. Found: 454.2777.

Crystal Structure Determination for Compound 35. Compound **35** was recrystallized from hexane. A crystal of dimensions 0.2 × 0.4 × 0.5 mm was selected and mounted on a glass fiber by using epoxy resin. Unit cell dimensions, a = 10.7836 (23) Å, b = 14.2153 (28) Å, c = 16.9969 (33) Å, vol = 2605.4 (9) Å^3, were determined on a Nicolet P3/F diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). The material crystallized in space group $P2_12_12_1$ with four molecules of formula $C_{24}H_{41}NO_7$ (M_r 455.60) present in the unit cell (ρ_{calcd} = 1.16 g/cm^3). In all, 5632 reflections were measured by using θ–2θ scans to $2\theta_{max}$ = 100°. These were empirically corrected for absorption and averaged to 2673 unique reflections of which 2555 were observed ($F_o > 6.0\sigma(F_o)$). The structure was solved by direct methods and refined by using cascading block least squares techniques.[19] At convergence, with all non-hydrogen atoms refined anisotropically and hydrogen atoms included in idealized positions, the final residuals were R = 0.033, wR = 0.047 for observed data and R = 0.035, wR = 0.054 for all data. There are no significant features in the final difference Fourier map.

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Supplementary Material Available: Tables of final atomic and positional parameters, atomic thermal parameters, and bond distances and angles for $C_{24}H_{41}NO_7$ (8 pages). Ordering information is given on any current masthead page.

Oxidation of β-Anilinoacrylate Alkaloids Vincadifformine and Tabersonine by Fremy's Salt. A Mechanistic Insight into the Rearrangement of *Aspidosperma* to *Hunteria* Alkaloids

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β-Anilinoacrylate *Aspidosperma* alkaloids vincadifformine (**2a**) and tabersonine (**2b**) react with Fremy's salt in aqueous acidic conditions via radical coupling at C-16. The resulting zwitterionic compounds **7a** and **10** rearrange to isoxazolidines **8** and then ultimately to azepino[2,3-*b*]indoles **9**. The mechanism of these reactions is discussed, and the structures of **7a**, **8b**, and **9a** were established by single-crystal X-ray analysis. Diazotization of amine **20b** (X = NH_2) affords fragmentation–cyclization products corresponding to eburnanes **18** and **21**. This reaction mimics the skeletal rearrangement of *Aspidosperma* → *Hunteria* alkaloids, and these findings support Wenkert's biogenetic proposal.

The monoterpenoid indole alkaloids represent an area of the wealth of natural products that continues to be a source of fascinating and informative investigations. In recent years, the chemistry of these compounds has elicited considerable synthetic interest and mechanistic scrutiny and such studies have been particularly important in development of a comprehensive rationalization of the relationship between different alkaloid skeleta. Moreover,

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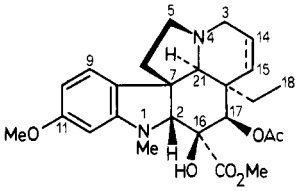
particular attention has been devoted to *Aspidosperma* alkaloids, and several elegantly conceived efforts have led to a formidable array of strategies for the synthesis of these compounds.[1]

Recently, we have reported[2] the synthesis of vindoline (VDL) (1), the dihydroindole portion of the potent oncolytic dimeric alkaloid vinblastine (VBL), starting from the largely available tabersonine (TBS) (**2b**) or its 11-methoxy derivative[3] via **3** (X = OH) by virtue of a regio- and stereoselective sequence. We find that the hydroxy group



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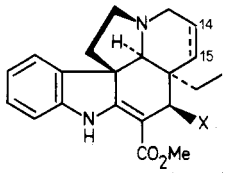
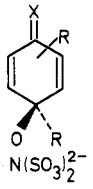
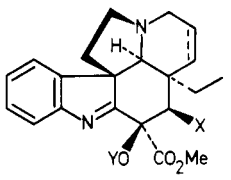


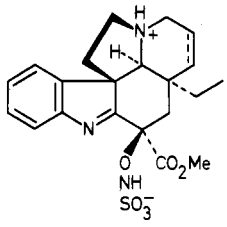
2a X: H
2b X: H Δ^{14}
3 X: OH, Nu



6 X: OH, NH



4a X,Y: H
4b X,Y: H, Δ^{14}
5 X: OH, Nu; Y: H
11a X: H; Y: NH_2
11b X: H; Y: NH_2; Δ^{14}
15a X: H; Y: N=CMe_2
15b X: H; Y: N=CMe_2; Δ^{14}



7a
7b Δ^{14}

in **3** (X = OH) is highly prone to nucleophilic displacement at C-17 to give **3** (X = Nu) under mild conditions in the presence of a wide variety of nucleophiles,[4] and our interest stems from the possibility of utilizing **3** (X = Nu) for the synthesis of biologically interesting 17-modified analogues of VDL. A critical step in the aforementioned approach is the hydroxylation of **3** at C-16 through the use of electrophilic oxidizing agents (e.g., *m*-chloroperoxybenzoic acid or lead(IV) acetate). This reaction is feasible under strictly controlled conditions, but, as noted also by Kuehne,[5] because of difficulty in controlling the extent of oxidation, it is not (at least as yet) a reliable and efficient process. This same shortcoming plagues the required hydroxylation of **3** (X = Nu), and consequently the need for an alternate oxidizing agent is manifest.

To this end, we thought that compounds of type **4** and **5** should be accessible from **2** and **3** after a free-radical C–O bond forming reaction, and we envisioned that nitrosodisulfonate radical anion $^{\bullet}ON(SO_3)_2^{2-}$,[6] a mild oxidant known as early as 1845, might be an attractive candidate for selective hydroxylation.

This paper, though failing in its initial concept, highlights the ability of β-anilinoacrylate alkaloids belonging to the *Aspidosperma* class, typified by vincadifformine (VDF) (**2a**) and TBS (**2b**), to undergo deep-seated skeletal reorganization and, meanwhile, discloses a novel facet of the manifold reactivity of these important alkaloids.

Results and Discussion

The nitrosodisulfonate radical anion represents one of the simplest and most long lived nitroxyl radicals which has found diversified application including much use as an ESR standard and a selective oxidizing agent. This radical is formed by virtually complete homolysis of its commercially available dimer, Fremy's salt (FS), when the latter is dissolved in polar solvents (eq 1). Reaction of

$$4K^+,[(O_3S)_2NO\text{–}ON(SO_3)_2]^{4-} \rightarrow 2[^{\bullet}ON(SO_3)_2]^{2-} + 4K^+ \quad (1)$$

phenols and aromatic amines with FS to quinoid compounds is well-documented and provides a useful synthetic tool for the introduction of an oxygenated function para or ortho to OH or NH groups. In this respect and consistent with the observed stoichiometry, the oxidation is thought to proceed through a mechanism in which the initially formed phenoxyl or amidyl radicals (by hydrogen abstraction) are scavenged in site-selective fashion (as predictable by FMO theory) to give intermediates of type **6**, which would then hydrolyze to final compounds (e.g., quinones, quinols, quinone imines).[7] With the aforementioned information in mind, it was expected that the β-anilinoacrylate moiety in **2** and **3** might easily generate, by interaction with FS, the amidyl radical A in which the unpaired electron is delocalized over a conjugated network. This intermediate would lead ultimately to the requisite compounds **4** and **5**. It is well-known that the site of reactivity of ambident radicals such as A should be largely determined by the coefficient of the SOMO or product-development control.[8] In A there are several sites that may, in principle, act as the coupling termini for FS (e.g., N-1, the ortho and para carbon atoms of the aromatic ring, and the carbon β to the N-1). On the other hand, it is intuitive that the presence of a CO_2Me group on C-16 should increase the relative importance of the resonance hybrid B vs A or C, thereby facilitating coupling of C-16 with the electrophilic nitroxyl radical. Finally, we visu-

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(4) Danieli, B.; Lesma, G.; Palmisano, G., unpublished results.

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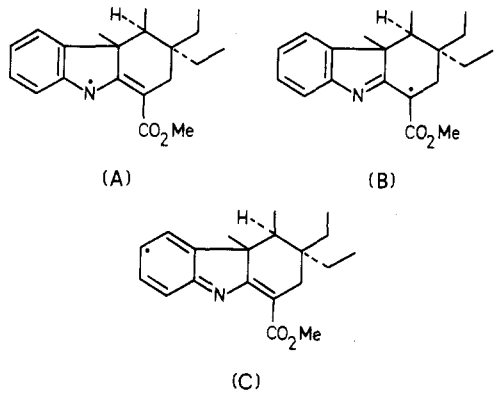
(6) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

(7) Recently, Castedo et al. suggested that nitrogen-centered radical cations (aminium radicals) were involved in the FS oxidation of some isoquinoline alkaloids: Castedo, L.; Puga, A.; Saa', J. M.; Suau, R. *Tetrahedron Lett.* **1981**, *22*, 2233.

(8) Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; Wiley-Interscience: London, 1976; pp 195–207.



alized that the attack of FS could be favored from the side opposite to the bulky ethyl chain at C-20 and could thus provide stereochemical control of the reaction to give β-hydroxylation.

Accordingly, VDF (**2a**) was selected as a model compound and reacted with FS under several sets of homogeneous conditions, but only starting material and a trace of minor side compounds were isolated, nor was any success to be found in using FS in a two-phase dichloromethane–water system in the presence of Adogen 464.[9] Conversely, exposure of **2a** to FS (4 equiv) in a H_2O–AcOH–DMF mixture (19:1:1) in the dark at ambient temperature met with success, giving **7a** as the sole compound (82% yield). Within 2 h we observed the gradual fading of the initially purple solution to yield an almost colorless suspension, and the isolation of zwitterionic **7a** was facilitated by its fortuitous insoluble nature in the reaction medium.[10] Initial studies using electron-impact mass spectrometry (EIMS) failed to give molecular weight information on **7a** owing to extensive decomposition; the positive fast atom bombardment mass spectrometry ($FABMS^+$) technique[11] (glycerol as matrix, 7-keV xenon atoms as primary beam) enabled the detection of pseudo molecular ion MH^+ at m/z 450 accompanied by the less abundant MNa^+ (m/z 472). Sequential losses of SO_3, SO_3NH, and SO_3NHO from MH^+ gave ions at m/z 370, 355, and 339, respectively, and the corresponding metastable peaks at m/z 304.2, 280.0, and 255.4 were also observed. The IR (KBr) spectrum exhibited a strong carbonyl band at 1740 cm^{-1} (vs 1670 cm^{-1} in VDF (**2a**)) while the UV spectrum (229 and 279 nm) suggested an indolenine chromophore. The 80-MHz ^1H NMR (CD_3OD) spectrum of **7a** showed a three-proton singlet at 3.84 ppm for CO_2Me whereas the four-proton multiplet between 7.20 and 7.40 ppm for aromatic protons had a complexity normally exhibited by authentic indolenines. Also present were two mutually coupled signals at 2.58 ppm (dd, J = 17.1, 1.4) and at 3.11 ppm (d, J = 17.1) due to diastereotopic protons at C-17 as well as a doublet (J = 1.4) at 3.87 ppm for H-21. As might be expected, protonation of the bridgehead N-4 influenced the chemical shift of the adjacent H-21, which displayed a low-field shift in relation to the unprotonated compounds.

Unfortunately, the stereochemical features of **7a** could not be ascertained with confidence on the basis of MS and ^1H NMR data alone. Consequently, recourse was made

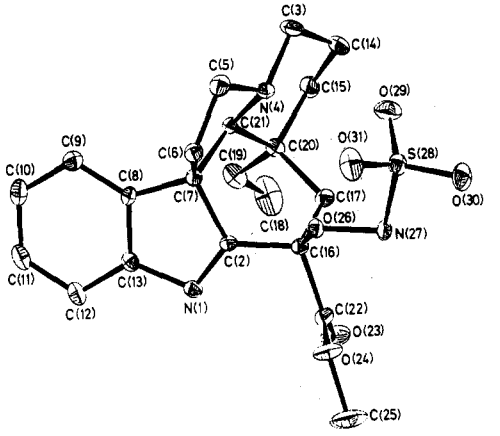
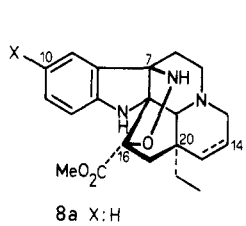


Figure 1.

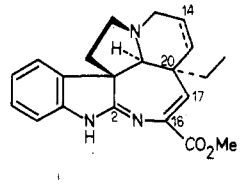
to single-crystal X-ray diffraction analysis of suitable crystals of **7a** grown from MeOH. The ORTEP diagram of Figure 1 discloses that **7a** is, as anticipated, stereochemically related to 16β-OH-VDF (**4a**), i.e., with the incoming oxygenated group and the ethyl chain at C-20 in an anti relationship. In a number of oxidations with FS, unstable intermediates have been isolated and ascribed to *O*-alkylhydroxylamine sulfonate derivatives,[12] but, to the best of our knowledge, this is the first authenticated example of such compounds.

Having readily reached our first goal, we needed to effect N–O bond cleavage in **7a**, and this operation could conceivably be accomplished by either reductive or hydrolytic treatment. However, despite numerous attempts under a variety of conditions, interference from either C=N double bond or N-4 lone pair cooperativity (retro-Mannich reaction) proved to be a stumbling block in our synthetic plan as well, since **7a** could not be cleanly converted to **4a** either.

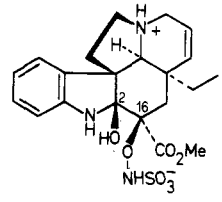
This study was undertaken because of the serendipitous finding that, although crystalline **7a** was stable for several months, it is somewhat prone to decomposition under acidic conditions. Thus, treatment of **7a** in 1 N hydrochloric acid at 70 °C for 90 min provided a reproducible mixture of isoxazolidine **8a** (68%) and azepino[2,3-*b*]indole derivative **9a** (25%).[13]



8a X:H
8b X:H; Δ^{14}
8c X:OH; Δ^{14}



9a
9b Δ^{14}



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(9) Olson, G. L.; Cheung, H.-C.; Morgan, K.; Saucy, G. *J. Org. Chem.* **1980**, *45*, 803.

(10) Different behavior of FS probably depends on the ability to change the $O\text{–}N(SO_3)_2^{2-}/HO\text{–}N(SO_3)_2^{2-}$ potential to a more positive value [0.801 V vs SCE (pH 5) vs 0.413 V (pH 12.5)] by changing pH to low values: Toropova, V. F.; Degtyareva, N. V. *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.* **1974**, *17*, 175; *Chem. Abstr.* **1974**, *80*, 152439g.

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(13) For leading references of azepino[2,3-*b*]indoles, see: Renfroe, B.; Harrington, C. In *Heterocyclic Compounds (Dibenzazepines and Other Tricyclic Azepines)*; Rosowsky, A., Ed.; Wiley: New York, 1984; Vol. 43, Part 1, pp 106–109.

The isoxazolidine **8a** showed a parent ion in the HR-EIMS consistent with a molecular formula of $C_{21}H_{27}N_3O_3$ and a prominent peak at m/z 338 ($C_{21}H_{26}N_2O_2$) by loss of HNO. The UV spectrum (246 and 297 nm vs 229 and 279 nm in **7a**) was characteristic of an indole chromophore. Weak Wenkert–Bohlmann bands at 2800 and 2745 cm^{-1} (*trans*-quinolizidine) and a carbonyl band at 1730 cm^{-1} were observed in the IR spectrum. The 200-MHz ^1H NMR spectrum of **8a** displayed four aromatic protons (7.23 (br dd, H-9), 7.10 (dt, H-11), 6.75 (dt, H-10), and 6.56 (dd, H-12) ppm), a broad singlet (1 H) at 2.31 ppm (H-21), and a triplet (J = 7.4) at 0.92 ppm for H-18. The stereochemistry at C-17 was revealed by the abnormally high chemical shift of the CO_2Me group (3.34 ppm vs 3.84 ppm in **7a**), and this upfield shift is a direct result of the CO_2Me lying in the shielding zone of the indoline π system. The noise-decoupled ^{13}C NMR spectrum of **8a** contained, inter alia, seven quaternary carbon atoms. Comparison of their chemical shifts with those of related indolines[14] showed that the ring A remained intact and therefore the resonances at 130.0 and 148.6 ppm are due to C-8 and C-13, respectively. Confirmation that modification occurred at C-2 and C-7 came from the disappearance of these signals in the 40–80-ppm region and the appearance of two new resonances at lower field (93.1 and 101.3 ppm) in **8a**. Even assuming that the structure of **8a** could be derived by rearrangement of **7a** with the preservation of the N–O bond, there were too many possibilities for the compound to be interpreted by analysis of spectral data alone. Initially, attempts were made to settle this question through X-ray crystallographic analysis, but we failed to produce suitable crystals. The assigned structure **8a** had been unambiguously confirmed by chemical correlation, and mechanistic evidence bearing on this point will be disclosed subsequently.

The second more polar product **9a** was fully characterized by spectral data and single-crystal X-ray diffraction study. HR-EIMS secured the formula of $C_{21}H_{25}N_3O_2$, differing from that of VDF (**2a**) by 15 amu, while the presence of an extended chromophore (232, 255, 287, 297, and 324 nm) followed from the electronic spectra. By means of homonuclear decoupling experiments, it was possible to assign the chemical shifts and coupling constants of most of the protons, except for H-14 and H-15 (see Experimental Section). The 200-MHz ^1H NMR spectrum of **9a** resembled that of VDF (**2a**), except that no H-17 could be observed at their usual positions and instead a low-field signal (6.24 ppm) appeared. Further support for structure **9a** was derived from the ^{13}C NMR data which were obtained from broad-band proton-decoupled and single-frequency nuclear Overhauser effect (NOE) spectra. The multiplicities of the different ^{13}C resonances were determined by generating the proton-decoupled CH, CH_2, and CH_3 subspectra by using the DEPT pulse technique.[15] Comparison of these data with those of **2a** showed that the only significant changes are for signals of carbon atoms in the region of ring C; more specifically, of the 10 signals in the 100–180-ppm region, seven corresponded by their shifts and multiplicities to the C's embodied in ring A and the CO_2Me group, while the other three (127.7 (d), 144.2 (s), and 164.7 (s) ppm) were assigned to C-17, C-16, and C-2, respectively. The structure **9a** was confirmed subsequently by a single-crystal X-ray diffraction analysis of the hydrobromide, and its ORTEP drawing is shown in Figure 2.

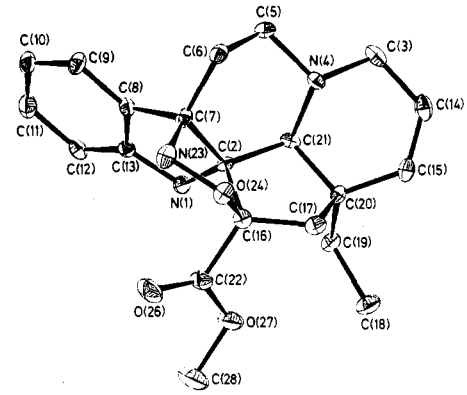


Figure 2.

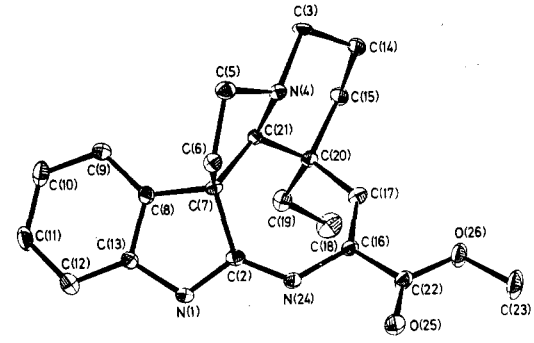


Figure 3.

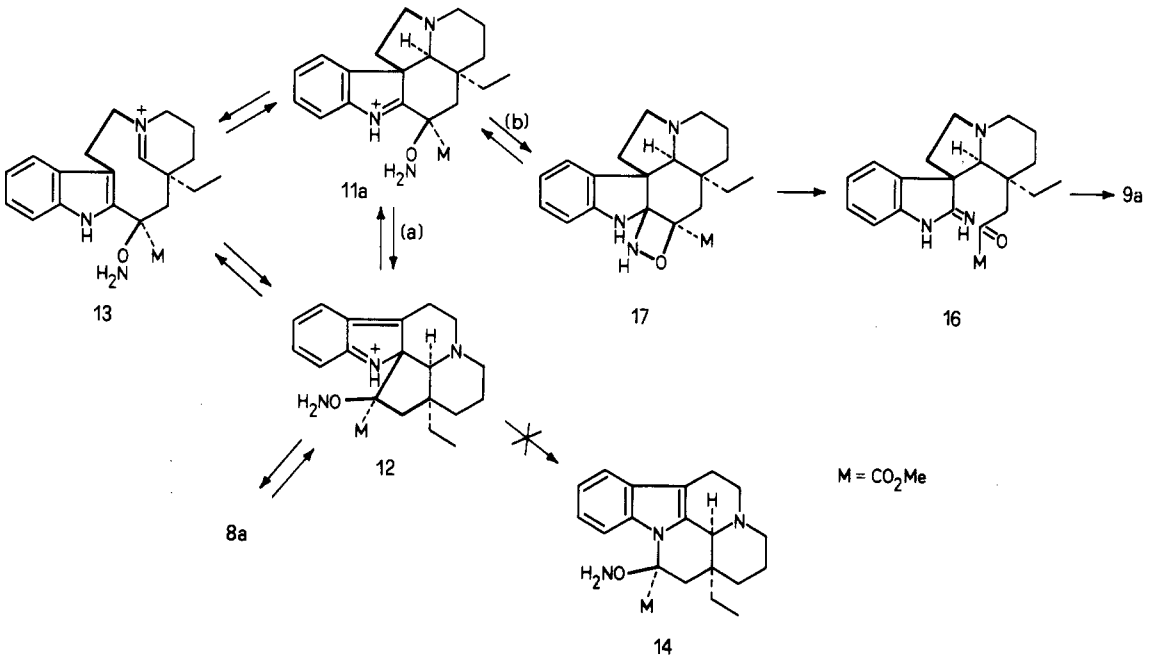
In order to verify the exact order of steps for the conversion of **7a** into **8a** and **9a**, we followed the fate of the starting material in a medium adequately conducive to rearrangement. Thus, on standing in 0.1 N aqueous trifluoroacetic acid at room temperature, **7a** was converted cleanly and completely (90 h) to **8a** and this, in turn, was gradually changed into **9a** (70 °C, 2 h) in 78% yield. A reaction period longer than 8 h decreased the yield of **9a** because of its partial decomposition under these reaction conditions, and the characterization of this mixture was not pursued.

To gain further insight into the mechanism of these reactions, we repeated the same sequence of events with TBS (**2b**) in the presence of FS. Surprisingly, the first step of the sequence was extremely sluggish and exposure of **2b** to 4 equiv of FS for 2 h at room temperature in H_2O–AcOH–DMF returned a substantial quantity of unreacted **2b** (52%), together with a mixture of zwitterionic indoline **10** (i.e., covalent hydrate of the putative indolenine **7b**) (6%) and 16-aminooxy indolenine **11b** (27%). Both these compounds are particularly susceptible to acid-induced rearrangement. Although a rigorous kinetic study was not undertaken, the transformation of zwitterions **7a** vs **10** in the same reaction medium (5% aqueous AcOH) was observed to proceed at distinctly different rates. Accordingly, the rearrangement of **10** was found to be complete (TLC) in ca. 2 h while the same reaction on **7a** was somewhat more difficult, requiring ca. 29 days (!) to reach completion at the same temperature.[16]

When the reaction mixture of TBS with FS was allowed to stand at ambient temperature in the dark for 4 days, a two-component mixture consisting of isoxazolidine **8b** (78%) (HR-EIMS, m/z 367.1896, calcd for $C_{21}H_{25}N_3O_3$ 367.1892; IR 3600, 2800, 2740, 1730 cm^{-1}) and its overox-

(14) Shamma, M.; Hindenlang, D. M. *Carbon-13 NMR Shift Assignments of Amines and Alkaloids*; Plenum: New York, 1979.
(15) Doddrell, D. M.; Pegg, D. T.,; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(16) The remarkable reactivity difference between **7a** and **10** might stem from subtle conformational changes.

Scheme I



idized derivative **8c** (8%) was cleanly formed. Comparison of the selectively decoupled ^{13}C NMR and the ^{1}H NMR (300 MHz) spectra allowed the complete assignment of **8b** (see Experimental Section). Although these data clearly revealed **8b** strictly related to **8a**, unequivocal structural definition could not be arrived at on this basis alone. Consequently, the monohydrobromide of **8b** was prepared and identified by X-ray crystal structure analysis (Figure 3). Apparently, skeletal reorganization of **10**/**11** (yielding **8b**) competes with subsequent ring hydroxylation resulting in formation of **8c**. Treatment of **8b** with FS under conditions identical with those for TBS and VDF led, by way of an intermediate quinone imine followed by reductive workup (aqueous sodium dithionite), exclusively to 10-hydroxy isoxazolidine **8c** in 85% yield.

All of the acid-catalyzed reactions of zwitterion **7a** can be fitted nicely into a common pattern (Scheme I). The mechanistic rationalization is based on the premise that the indoleninium cation **11a**, arising as a primary hydrolysis product of **7a**, may suffer a formal C(2) → C(7) alkyl shift leading to the 21(7→2)-abeoaspidospermane intermediate **12**. On the other hand, this migration can be interpreted as a [1,5] sigmatropic rearrangement or the result of a retro-Mannich reaction to give the *chano*-iminium cation **13**, followed by Mannich condensation at the indole α-position C-2. The former rearrangement pathway is analogous to previous examples in which 3,3-disubstituted indolenines undergo facile Wagner–Meerwein rearrangement to yield 2,3-disubstituted indoles.[17]

The resonance-stabilized cation **12** is then intramolecularly trapped by the suitably positioned nucleophilic hydroxylamine nitrogen in a stereospecific fashion to give **8a** rather than regain aromaticity via a [1,5] shift leading to the eburnane derivative **14**. Although **11a** cannot be isolated under the various acid-catalyzed decomposition conditions used for **7a**, its existence can be inferred from the fact that the analogous compound **11b** was isolated, albeit in poor yields, from FS oxidation of TBS (**2b**). In the last case, control experiments showed that **11b** (and hence **10**) and **8b** are readily interconvertible, and this equilibrium was found to lie almost completely on the isoxazolidine side. However, we succeeded in intercepting quantitatively the indolenine **11a** as oxime **15a** by treatment of **8a** with acetone in the presence of a catalytic amount of anhydrous cooper(II) sulfate or phosphomolybdic acid. Treatment of **15a** in 0.1 N HCl–THF (1:1) for 2 h at room temperature resulted in the reverse reaction, i.e., cleavage of the oxime and reformation of isoxazolidine **8a**.

With respect to a possible pathway to the azepine **9a**, it could be initiated by the conversion of **11a** (and hence of isoxazolidine **8a**) into the open-ring intermediate **16** by intramolecular nucleophilic attack to give the predictably elusive 1,2-oxazetidine **17**, followed by its formal [σ2s + σ2a] cycloreversion. This mechanism has close similarity to that proposed by Levy et al.[18] for the C-2–C-16 oxidative cleavage of VDF and its 3-oxo derivative. Intramolecular condensation of the exocyclic amidine nitrogen with the most electrophilic α-carbonyl group of the methoxalyl moiety in **16** (*7-Exo-Trig* ring closure) would lead ultimately to the azepine **9a**.[19] As a consequence of the irreversibility of the N–O bond cleavage, the facile formation of **16** shifts the whole set of equilibria toward path B, and hence, **9a** represents the probable end point of the cascade.

The recognition by Wenkert[20] that the structure of eburnane alkaloids (*Hunteria*), typified by vincamine, could be rationalized in biogenetic terms as resulting from oxidative rearrangement of an *Aspidosperma* precursor was of fundamental importance to the development of subsequent biogenetic theories, and mimics of this biosynthetic pathway have inspired successful synthetic approaches to some of the *Hunteria* alkaloids.[21] As recog-

(17) (a) Jackson, A. H.; Naidoo, B.; Smith, P. *Tetrahedron* **1968**, *24*, 6119. (b) Jackson, A. H.; Naidoo, B. *Tetrahedron* **1969**, *25*, 4843.

(18) Hugel, G.; Laronze, J.-Y.; Laronze, J.; Levy, J. *Heterocycles* **1981**, *16*, 581. See also: Calabi, L.; Danieli, B.; Lesma, G.; Palmisano, G. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1371.

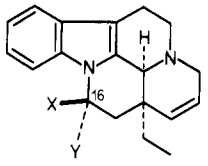
(19) While our study was in progress, Lewin and Poisson reported the isolation of a hydroxamic acid bearing the azepino[2,3-*b*]indole ring system (aza-*C*-homoaspidospermane) from TFA treatment of 16α-nitro-2,16-dihydro-VDF via a Beckmann-like rearrangement: Lewin, G.; Poisson, J.; Toffoli, P. *Tetrahedron* **1987**, *43*, 493.

(20) Wenkert, E.; Wickberg, B. *J. Am. Chem. Soc.* **1965**, *87*, 1580.

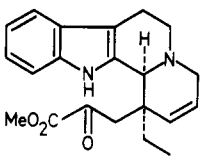
(21) (a) Hugel, G.; Levy, J.; Le Men, J. *Tetrahedron Lett.* **1974**, 3109. (b) Croquelois, G.; Kunesch, N.; Poisson, J. *Tetrahedron Lett.* **1974**, 4427. (c) Danieli, B.; Lesma, G.; Palmisano, G.; Gabetta, B. *J. Chem. Soc., Chem. Commun.* **1981**, 908.

nized by Wenkert and later, in part, confirmed by Le Men and Levy,[22] an indoleninium cation such as **19**, having a 21(7→2)-abeoaspidospermane skeleton, might act as a connecting link between the *Aspidosperma* and *Hunteria* alkaloids. To our knowledge, however, there are no published reports of the unequivocal synthesis of **19** (through its synthetic equivalent **20** with X as nucleofugal group); consequently, the problem of whether the *Aspidosperma* → *Hunteria* interconversion occurs via **19** remains an open question. The isolation of several alkaloids (e.g., vallesamidine[23] and andrangine[24]) embodying this skeletal arrangement lent further support to Wenkert's original biogenetic proposal.

Encouraged by the ready accessibility of isoxazolidines **8a** and **8b**, we reasoned that the diazonium cation **20** (X = N_2^+), available through N–O reductive cleavage and subsequent diazotization, could serve equally well as a precursor to **19**. Accordingly, treatment of **8b** with a large excess of Zn dust in AcOH at room temperature cleanly produced the requisite amine **20b** (X = NH_2) (89%). Subsequent exposure of **20b** (X = NH_2) to *tert*-butyl nitrite at 0 °C in THF containing AcOH yielded, via the corresponding diazonium salt and concomitant ejection of N_2, a separable mixture of 14,15-dehydrovincamine (**18**) and its 16-epi derivative **21** in a 1:1 ratio. This ratio remained unchanged on longer reaction time and was reproducible. Notably, an essentially identical **18**/**21** ratio was observed by acid-induced rearrangement of 16β-OH-TBS (**4b**), generated in situ by deoxygenation of the corresponding *N*-oxide.[25] It would therefore appear that the two substrates, i.e., **4b** and **20b** (X = N_2^+), react via one and the same intermediate **19**. Even though [1,5] sigmatropic shift involvement cannot be ruled out in the subsequent rearrangement of **19** to **18**/**21**, the lack of stereospecificity observed in these reactions strongly suggested a stepwise mechanism.[26] Apparently, the HO–C-16 participates in the stereoselectronically allowed breakdown of ring E to relieve ring strain (with concomitant aromatization of ring B) leading to an α-keto ester **22**, which then partitions itself to give the thermodynamic mixture of **18** and **21**.

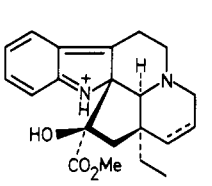


18 X:OH; Y:CO_2Me
21 X:CO_2Me; Y:OH

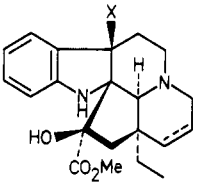


22

While studying the chemical behavior of **8a**, we observed that catalytic hydrogenation over 10% Pd–C at atmospheric pressure in MeOH brought about not only the saturation of the double bond but also the simultaneous cleavage of the N–O bond and benzylic hydrogenolysis to give **20a** (X = H). The same product was obtained from **8b** and **20b** (X = NH_2). The EIMS of **20a** (X = H) showed a strong peak at m/z 356 as well as satellite peak intensities compatible with a molecular formula $C_{21}H_{28}N_2O_3$. The UV spectrum gave absorption maxima at 246 and 294 nm, which are very similar to those seen in the UV spectrum of andrangine.[24] In the ^1H NMR spectrum of **20a** (X = H), the triplet signal (J = 7.0) at 0.90 ppm could be assigned to the C-18 methyl group, whereas the C-17 protons appeared as an AB system (J = 13.5) at 2.66 and 2.00 ppm.



19



20a – X: H, NH_2, N_2^+
20b Δ^{14}

X-ray Crystallography

Figures 1–3 show the perspective view (drawn by ORTEP II[27]) of **7a** hydrate (XR-1), isoxazolidine **8b** hydrobromide (XR-2) and azepine **9a** dihydrobromide (as methanol solvate, XR-3). These figures also carry the atomic numbering scheme adopted for crystallographic purposes (and used in the remainder of this section). Table I is a summary of crystallographic data, data collection, and refinement for the above-mentioned structures.

The molecule of XR-1 is a zwitterion (sulfobetaine): the acid hydrogen coming from SO_3H is well-localized on N(4), and the hydrogen bond N(4)–H(4)...O(29) is responsible for the conformation of the hydroxylamine-*N*-sulfonate arm. Another strong hydrogen bond is N(27)–H(27)...O(W), with N(27)...O(W) = 2.925 (8) Å, and the crystal packing is based on a network of hydrogen bonds involving hydroxylamine-*N*-sulfonate and carboxy groups and O(W).

In the crystal structure of XR-2, we observed three bonds, e.g., C(2)–C(7) [1.577 (7) Å], C(2)–C(16) [1.578 (7) Å], and C(2)–N(1) [1.459 (7) Å], which are significantly longer than the analogous ones in XR-1 and XR-3. XR-2 is also characterized by the presence of two intramolecular hydrogen bonds: the strong N(23)–H(23)...O(26) with N(23)...O(26) [2.913 (7) Å] and the weaker N(1)–H(1)...O(27) with N(1)...O(27) = [3.207 (6) Å]. Conversely, apart from N(4)–H(4)...Br, there are few weak intermolecular hydrogen bonds, and the crystal packing is influenced by van der Waals interactions much more than in XR-1 and XR-3.

Finally, XR-3 did not appear particularly strained except for C(6)–C(7) [1.564 (9) Å], which is slightly elongated with respect to the usual C(sp^3) bond length. The benzene ring, the chain N(1)–C(2)–N(24)–C(16)–C(17), and the CO_2Me group form a well-conjugated π system. In fact, the maximum angle between couples of p_z orbitals along the whole system is 20.3 (9)° for N(24)–C(16) while the N(1)–C(2) single bond and the C(2)–N(24) double bond have similar lengths [1.327 (7) Å and 1.310 (7) Å, respectively]. Crystal packing of XR-3 is essentially governed by electrostatic interactions among the heteroatoms.

Conclusions

From the synthetic standpoint, the results reported here are not encouraging; however, the reaction of the β-ani-

(22) (a) Mauperin, P.; Levy, J.; Le Men, J. *Tetrahedron Lett.* **1971**, 999. (b) Mauperin, P.; Doe de Maindreville, M.; Le Men, J. *Tetrahedron Lett.* **1971**, 1003. (c) Hajicek, J.; Trojanek, J. *Collect. Czech. Chem. Commun.* **1986**, *51*, 1731.

(23) (a) Brown, S. H.; Djerassi, C.; Simpson, P. G. *J. Am. Chem. Soc.* **1968**, *90*, 2445. (b) Walser, A.; Djerassi, C. *Helv. Chim. Acta* **1965**, *48*, 391.

(24) Kan-Fan, C.; Das, B. C.; Husson, H.-P.; Potier, P. *Bull. Soc. Chim. Fr.* **1974**, 2839.

(25) Hugel, G.; Gourdier, B.; Levy, J.; Le Men, J. *Tetrahedron* **1980**, *36*, 511.

(26) A [1,5] sigmatropic change in the suprafacial mode should proceed with net retention of configuration of the migrating group C-16. Compare: Hugel, G.; Levy, J. *Tetrahedron* **1984**, *40*, 1067.

(27) Johnson, C. K. *ORTEP II*; Report ORNL 5138; Oak Ridge National Laboratory, Oak Ridge, TN.

Table I. Details of Crystallographic Data, Data Collection, and Structure Refinement for XR-3, XR-1, and XR-2[a]

	XR-3	XR-1	XR-2
formula weight	567.54	545.36	444.36
system	monoclinic	orthorhombic	orthorhombic
space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a, Å	7.834 (2)	8.829 (2)	10.939 (2)
b, Å	19.129 (2)	10.815 (2)	12.004 (3)
c, Å	8.629 (2)	23.694 (4)	15.625 (3)
β, deg	110.06 (2)	–	–
V, Å^3	2051.8	2262.4	1214.7
Z	2	4	4
D_x, g cm^{-3}	1.491	1.370	1.452
radiation		Mo $K\alpha$, λ = 0.71069 Å	
μ, cm^{-1}	33.3	20.1	1.8
F(000)	566	988	928
cell-parameter determination			
θ range of reflection, deg	17–18	12–14	14–16
no.	25	25	25
data collection			
range h	0, 10	0, 9	0, 14
range k	0, 24	0, 11	0, 15
range l	−11, 11	0, 25	0, 20
range θ	0, 27.5	0, 22.5	0, 27.5
no. of check reflections	3	3	3
variation of intensity	<3%	<2%	<2%
range of absorption correction	0.94–1.00	–	–
no. of unique reflections collected	2864	1722	2660
refinement			
no. of refined reflections [if $I > \sigma(I)$]	2518	1457	2224
R	0.047	0.039	0.044
R_w	0.038	0.041	0.035
weighting scheme: $w = 4I_o/[\sigma^2(I_o) + sI_o^2]$, s	0.0004	0.0004	0.0004
Δ/σ_{max} in the last cycle	0.10	0.05	0.02
$\Delta\rho$ range in final difference Fourier map (e Å^{-3})	−0.2, 0.5	−0.2, 0.4	−0.2, 0.3
extinction coefficient g (×10^6)	–	8 (1)	4 (1)

[a] All measurements were performed on a Nonius CAD4 diffractometer.

linoacrylate *Aspidosperma* alkaloids, vincadifformine (**2a**) and tabersonine (**2b**), with Fremy's salt offers the opportunity to observe unprecedented reaction-mechanism channels and to shed some light on the labyrinth of interconnecting pathways between different alkaloid families.

Further oxidation studies with FS on related systems (e.g., 17-substituted tabersonines) are also underway and will be the subject of a future report.

Experimental Section

Melting points are uncorrected and were determined in open-ended capillaries. Infrared spectra were obtained on a Perkin-Elmer 681 spectrophotometer, and ultraviolet spectra are for solutions in MeOH on a Perkin-Elmer 554 UV–vis spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker WP-80 (80 MHz), Varian XL-200 (200 MHz), or Bruker CXP-300 (300 MHz) instrument in $CDCl_3$ unless otherwise stated. ^{13}C NMR spectra were obtained on a Varian XL-200 (50.4 MHz) instrument. Chemical shifts are expressed in parts per million downfield from internal Me_4Si, and coupling constants (J values) are given in hertz. Mass spectra (EI and positive FAB) were recorded on a VG 70-70 EQ instrument operating at 70 eV. Compounds were detected on developed chromatograms by fluorescence quenching (λ 254 or 365 nm) or visualized with cerium(IV) ammonium sulfate (CAS, 1% in 85% phosphoric acid); R_f and colors (CAS spray on TLC (silica gel)) of products are given. Solvent systems: (A) diethyl ether; (B) diethyl ether saturated with concentrated ammonia; (C) benzene–EtOH–concentrated ammonia, 89:10:1; (D) $CHCl_3$–MeOH–concentrated ammonia, 70:30:2. Flash chromatography (FC) was carried out as described by Still et al.[28] and performed with silica gel S (230–400 mesh). Preparative TLC was performed on 1 mm thick layers of Merck silica gel HF_{254} coated on 20 × 20 cm glass plates.

Reaction of VDF (2a) with Fremy's Salt. To a solution of dipotassium nitrosodisulfonate (FS) (1.58 g, 5.92 mmol) in a water–AcOH mixture (19:1) (60 mL) was added a solution of VDF (**2a**) (500 mg, 1.48 mmol) in DMF (3 mL), and the mixture was kept at room temperature in the dark for 2 h, during which time the intense purple color of FS had disappeared and a white solid crystallized out. After cooling at 10 °C, the solid was filtered and proved to be a single compound (TLC). This highly insoluble material was recrystallized from hot MeOH to give pure **7a** (545 mg, 82%) as colorless crystals: mp 205 °C dec; R_f (D) 0.73 (orange); UV 229 and 279 nm; IR (KBr) 3610, 3430, 1745 cm^{-1}; ^{1}H NMR (80 MHz, CD_3OD) δ 7.68–7.23 (m, 4 H, H aromatic), 3.84 (s, OMe), 3.78 (d, J = 1.4, H-C(21)), 3.11 (d, J = 17.1, β-H-C(17)), 2.58 (dd, J = 17.1, 1.4, α-H-C(17)), 0.43 (t, J = 6.8, H-C(18)); FABMS, m/z 472 (MNa^+), 450 (MH^+), 370 ($MH^+ - SO_3$), 355 ($MH^+ - SO_3NH$), 339 ($MH^+ - SO_3NHO$).

Acid-Catalyzed Rearrangement of 7a to Isoxazolidine 8a and Azepine 9a. A slurry of zwitterion **7a** (350 mg, 0.78 mmol) in 1 N HCl (15 mL) was heated under nitrogen to 70 °C for 90 min, with concomitant TLC analysis. After cooling to room temperature, water (50 mL) was added and the mixture made basic with concentrated ammonia and extracted with $CHCl_3$ (15 mL, 3 times). The organic extract was washed with water (20 mL, 3 times) and then with brine (20 mL). After drying ($MgSO_4$), it was concentrated in vacuo and the residue purified by FC (95.5:0.5 Et_2O–concentrated NH_3), giving the isoxazolidine **8a** (195 mg, 68%) and the azepine **9a** (68 mg, 25%).

8a: R_f (B) 0.71 (red); UV 246 and 297 nm; IR ($CHCl_3$) 3410, 2800, 2745, 1730 cm^{-1}; ^{1}H NMR (200 MHz) δ 7.23 (br dd, J = 7.7, 1.3, H-C(9)), 7.12 (br s, N(1)-H), 7.10 (dt, J = 7.7, 1.7, H-C(11)), 6.75 (dt, J = 7.7, 1.3, H-C(10)), 6.56 (dd, J = 7.7, 1.7, H-C(12)), 4.35 (br s, ONH), 3.44 (br s, OMe), 2.31 (br s, H-C(21)), 0.92 (t, J = 7.4, H-C(18)); ^{13}C NMR δ 173.5 (C=O), 148.6 (C-13), 130.0 (C-8), 129.4 (C-11), 123.7 (C-9), 119.6 (C-10), 109.8 (C-12), 101.3 (C-7), 93.1 (C-2), 77.0 (C-16), 54.2 (C-5), 51.9 (C-3), 50.5 (C-20), 31.9 (C-15), 30.7 (C-6), 21.7 (C-14), 7.8 (C-18); HR-EIMS, m/z 369.2050 (calcd for $C_{21}H_{27}N_3O_3$, 369.2052).

9a: R_f (A) 0.37 (colorless); UV 232, 255, 287, 297, and 324 nm; IR ($CHCl_3$) 3370, 1715, 1705 cm^{-1}; ^{1}H NMR (200 MHz) δ 7.29–7.19 (m, 3 H, H aromatic), 7.03 (ddd, J = 7.6, 6.75, 2.2, H-C(10)), 6.24 (d, J = 1.5, H-C(17)), 3.83 (s, OMe), 3.24 (ddt, J = 11.2, 4.4, 1.9,

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H_S-C(3)), 3.09 (dd, J = 9.0, 5.0, H_R-C(5)), 2.80 (d, J = 1.5, H-C(21)), 2.63 (ddd, J = 11.2, 9.0, 4.7, H_S-C(5)), 2.40 (ddd, J = 11.2, 10.5, 3.0, H_R-C(3)), 2.21 (dt, J = 11.2, 6.0, H_S-C(6)), 1.53 (dd, J = 11.2, 4.7, H_R-C(6)), 1.04 (m, 2 H, H-C(19)), 0.60 (t, J = 7.2, H-C(18)); ^{13}C NMR δ 176.7 (C=O), 164.7 (C-21), 151.4 (C-13), 144.2 (C-16), 127.7 (C-17, C-11), 127.3 (C-8), 122.0 (C-9), 120.5 (C-10), 116.5 (C-12), 59.1 (C-7), 52.4 (OMe, C-5), 51.0 (C-3), 41.0 (C-6), 36.2 (C-15), 28.6 (C-19), 22.3 (C-14), 8.6 (C-18); HR-EIMS, m/z 351.1948 (calcd for $C_{21}H_{25}N_3O_2$, 351.1946).

When the reaction was carried out in 0.1 N TFA solution under nitrogen, the starting material was completely consumed in 96 h to yield pure **8a** (95%).

Reaction of TBS (2b) with Fremy's Salt. To a solution of Fremy's salt (470 mg, 1.76 mmol) in 19:1 water–AcOH (20 mL) was added a solution of TBS (**2b**) (150 mg, 0.44 mmol) in DMF (1 mL), and the resulting mixture was stirred at room temperature in the dark. After 2 h, the reaction mixture was diluted with water (50 mL) and exhaustively extracted with n-BuOH (15 mL, 3 times). The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated to dryness. Purification by preparative TLC ($CHCl_3$–MeOH–NH_3, 70:30:2) gave unreacted TBS (**2b**) (78 mg, 52%), the indolenine **11b** (44 mg, 27%), and the zwitterion **10** (12 mg, 6%).

11b: R_f (D) 0.35 (orange); UV 276 nm; IR ($CHCl_3$) 1735 cm^{-1}; ^{1}H NMR (80 MHz) δ 7.70–7.27 (m, 4 H, H aromatic), 5.70 (m, 2 H, H-C(14) and H-C(15)), 3.89 (s, OMe), 3.77 (br s, ONH_2), 2.72 (d, J = 15.2, H-C(17)), 0.88 (br q, J = 6.8, H-C(19)), 0.37 (t, J = 6.8, H-C(18)); EIMS, m/z 367 (M^+, 45), 352 (53), 336 (100). Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.84; H, 6.85; N, 11.43. Found: C, 68.98; H, 6.73; N, 11.56.

10: R_f (D) 0.23 (red); UV 242, 292 (sh), 300 nm; IR (KBr) 1740 cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.72 (m, H-C(14) and H-C(15)), 3.86 (s, OMe), 0.52 (t, J = 7.0, H-C(18)); FABMS, m/z 464 (MH^+), 384 (MH – SO_3), 369 (384 – NH); HR-FABMS, m/z 464.1487 (calcd for $C_{21}H_{26}N_3O_7S$, 464.1491).

The same reaction mixture was set aside at room temperature in the dark for 4 days, during which time the initial purple solution turned pale yellow. After addition of CH_2Cl_2 (50 mL), the organic phase was separated. Evaporation of the solvent followed by flash chromatography (Et_2O) gave the isoxazolidine **8b** (127 mg, 78%) and its 10-hydroxy derivative **8c** (13 mg, 8%).

8b: mp 134 °C (Et_2O); R_f (A) 0.29 (red); UV 244 and 298 nm; IR ($CHCl_3$) 3410, 2800, 2740, 1730 cm^{-1}; ^{1}H NMR (300 MHz) δ 7.21 (dd, J = 7.5, 1.2, H-C(9)), 7.19 (ddd, J = 7.5, 1.2, 1.1, H-C(11)), 6.89 (br s, N(1)-H), 6.82 (ddd, J = 7.5, 1.2, 1.1, H-C(10)), 6.63 (dd, J = 7.5, 1.1, H-C(12)), 5.80 (ddd, J = 10.0, 5.6, 1.2, H-C(14)), 5.62 (ddd, J = 10.0, 2.5, 1.2, H-C(15)), 4.17 (br s, NH), 3.66 (s, OMe), 3.21 (ddd, J = 16.0, 5.6, 1.2, H-C(3)), 2.72 (ddd, J = 12.0, 4.0, 0.5, H-C(5)), 2.71 (ddd, J = 16.0, 2.5, 1.2, H-C(3)), 2.61 (dd, J = 13.8, 1.0, H-C(17)), 2.59 (ddd, J = 13.5, 2.8, 0.5, H-C(6)), 2.49 (ddd, J = 13.5, 12.0, 4.9, H-C(6)), 2.32 (dd, J = 13.8, 1.0, H-C(17)), 2.29 (d, J = 1.0, H-C(21)), 1.92 (ddd, J = 12.0, 12.0, 2.8, H-C(5)), 1.74 (q, J = 7.4, H-C(19)), 1.00 (t, J = 7.4, H-C(18)); ^{13}C NMR δ 172.8 (C=O), 148.0 (C-13), 131.0 (C-15), 130.1 (C-11), 127.0 (C-8), 123.9 (C-14 and C-9), 119.1 (C-10), 109.7 (C-12), 102.3 (C-7), 92.9 (C-2), 77.0 (C-3), 76.2 (C-16), 54.7 (C-20), 53.2 (C-3), 51.9 (OMe), 49.8 (C-5), 43.1 (C-17), 35.5 (C-19), 30.0 (C-6), 8.7 (C-18); HR-EIMS, m/z 367.1896 (calcd for $C_{21}H_{25}N_3O_3$, 367.1894).

8c: R_f (A) 0.11 (red-scarlet); UV 320 and 240 nm; IR ($CHCl_3$) 3600, 3385, 2800, 1735 cm^{-1}; ^{1}H NMR (80 MHz) δ 6.80 (m, H-C(9)), 6.68 (dt, J = 7.8, 2.2, H-C(11)), 6.52 (dd, J = 7.8, 0.9, H-C(12)), 5.81 (ddd, J = 10.2, 4.5, 1.2, H-C(14)), 5.58 (br dd, J = 10.2, 1.45, H-C(15)), 3.94 (br s, NH), 3.63 (s, OMe), 3.23 (dd, J = 16.1, 4.5, H-C(3)), 2.71 (br d, J = 16.1, H-C(3)), 2.63 (dd, J = 13.3, 1.0, H-C(17)), 2.31 (s, H-C(21)), 2.30 (d, J = 13.3, H-C(17)), 1.74 (q, J = 7.3, H-C(19)), 0.99 (t, J = 7.3, H-C(18)); HR-EIMS, m/z 383.1847 (calcd for $C_{21}H_{25}N_3O_4$, 383.1845).

Reaction of Isoxazolidines 8a and 8b with Acetone. A solution of isoxazolidine **8a** (50 mg, 0.13 mmol) in dry acetone (5 mL) containing phosphomolybdic acid (5 mg) was stirred under nitrogen at room temperature for 3 h. The reaction mixture was evaporated to provide a green residue, which was taken up in water (50 mL) and extracted with CH_2Cl_2 (15 mL, 2 times). The combined organic layers were dried ($MgSO_4$) and concentrated to give a solid, which was purified by preparative TLC (Et_2O) to give oxime **15a** (51 mg, 92%) as a colorless foam: R_f (A) 0.67 (orange); UV 224 and 274 nm; IR ($CHCl_3$) 2800, 2770, 1740 cm^{-1}; ^{1}H NMR (80 MHz) δ 7.65–7.15 (m, 4 H, H aromatic), 3.87 (s, OMe), 2.92 (d, J = 14.4, β-H-C(17)), 2.49 (dd, J = 14.4, 1.3, α-H-C(17)), 2.45 (d, J = 1.3, H-C(21)), 1.91 and 1.89 (2 s, NMe); FABMS, m/z 410 (MH^+). Anal. Calcd for $C_{24}H_{31}N_3O_3$: C, 70.39; H, 7.53; N, 10.26. Found: C, 70.85; H, 7.69; N, 10.32.

Using the procedure described above for the synthesis of **15a**, we obtained oxime **15b** in 89% yield by starting from **8b**. **15b**: R_f (A) 0.69 (orange); UV 224 and 270 nm; IR ($CHCl_3$) 2800, 2780, 1740 cm^{-1}; ^{1}H NMR (200 MHz) δ 7.61 (dd, J = 7.1, 1.7, H-C(9)), 7.18–7.40 (m, 3 H, H aromatic), 5.71 (ddd, J = 9.7, 4.6, 1.5, H-C(14)), 5.46 (ddd, J = 9.7, 2.1, 2.0, H-C(15)), 3.89 (s, OMe), 3.54 (ddd, J = 15.5, 4.6, 2.0, H-C(3)), 3.30 (dd, J = 8.0, 6.5, H-C(5)), 2.99 (ddd, J = 15.5, 2.1, 1.5, H-C(3)), 2.97 (ddd, J = 12.0, 11.7, 6.5, H-C(6)), 2.87 (d, J = 14.4, H-C(17)), 2.79 (br s, H-C(21)), 2.71 (ddd, J = 12.0, 8.0, 4.8, H-C(5)), 2.69 (dd, J = 14.4, 1.5, H-C(17)), 1.89 and 1.87 (2 s, N=CMe_2), 1.68 (dd, J = 11.7, 4.8, H-C(6)), 1.05, 0.88 and 0.47 (ABM_3 system, J = 15.0, 7.4); ^{13}C NMR δ 181.9 (C-2), 172.5 (C=O), 156.5 (C=N), 152.7 (C-13), 134.2 (C-15), 147.6 (C-8), 127.0 (C-10), 126.7 (C-11), 122.0 (C-9), 120.9 (C-12), 84.1 (C-16), 73.1 (C-21), 60.5 (C-7), 54.1 (C-5), 52.4 (OMe), 52.0 (C-3), 40.3 (C-20), 34.5 (C-17), 27.7 (C-19), 21.9 (MeC=N), 16.0 (MeC=N), 8.1 (C-19); EIMS, m/z 351 (9), 335 (29), 305 (5), 121 (100). Anal. Calcd for $C_{24}H_{33}N_3O_3$: C, 70.04; H, 8.08; N, 10.21. Found: C, 70.35; H, 8.15; N, 10.32.

Reduction of Isoxazolidine 8b to 20b (X = NH_2). To a solution of **8b** (65 mg, 0.17 mmol) in AcOH (5 mL) under nitrogen was added in small portions Zn dust (0.5 g). The mixture was stirred for 8 h, during which time two more portions of Zn dust were added. The mixture was filtered through Celite, with washing successively with AcOH (5 mL) and $CHCl_3$ (10 mL). The solvents were removed in vacuo, and the residue was taken up in CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ solution (10 mL, 2 times), and dried ($MgSO_4$). After filtration, concentration in vacuo, and flash chromatography (C), amine **20b** (56 mg, 89%) was isolated as a colorless glass: R_f (C) 0.33 (violet); UV 244 and 293 nm; IR ($CHCl_3$) 3415, 1735 cm^{-1}; ^{1}H NMR (80 MHz) δ 7.19 (dd, J = 7.3, 1.8, H-C(9)), 7.06 (dt, J = 7.3, 1.8, H-C(11)), 6.88 (dt, J = 7.3, 1.8, H-C(10)), 6.81 (dd, J = 7.3, 1.8, H-C(12)), 5.82 (ddd, J = 10.0, 5.7, 1.6, H-C(14)), 5.54 (dd, J = 10.0, 2.2, H-C(15)), 5.21 (br s, N(1)-H), 3.75 (s, OMe), 3.00 (dd, J = 15.2, 5.7, H-C(3)), 2.91 (br s, ONH_2 and OH), 2.48 (ddd, J = 15.2, 2.2, 1.6, H-C(3)), 2.47 (d, J = 13.1, H-C(17)), 2.27 (br s, H-C(21)), 2.17 (br d, J = 13.1, H-C(17)), 0.73 (t, J = 7.1, H-C(18)); EIMS, m/z 369 (M^+, 5), 352 (19), 293 (10), 265 (25), 170 (100); FABMS, m/z 370 (MH^+).

Hydrogenation of Isoxazolidine 8a to 20a (X = H). Isoxazolidine **8a** (50 mg, 0.13 mmol) was dissolved in MeOH (5 mL) and hydrogenated in the presence of 10% Pd–C (10 mg) at 1 atm for 1 h. TLC analysis (C) revealed the disappearance of starting material and the presence of three new spots with R_f values of 0.45 (red; major component) and 0.40 and 0.35 (yellow) as minor components, respectively. The catalyst was filtered off, and the filtrate was evaporated to give a yellowish glass. Preparative TLC (silica, benzene–propan-2-ol–concentrated ammonia, 96:3:1) allowed the separation of the high-R compound **20a** (X = H) (24 mg, 52%) as a colorless foamy powder: UV 246 (3.86) and 294 (3.39) nm; IR ($CHCl_3$) 1735 cm^{-1}; ^{1}H NMR (80 MHz) δ 7.1–6.5 (m, aromatic protons), 3.86 (br s, N(1)-H, exchangeable with D_2O), 3.43 (s, CO_2Me), 2.66 and 2.00 (AB system, J = 13.5, diastereotopic H_2-C(17)), 2.33 (s, H-C(21)), 0.90 (t, J = 7.0, H-C(18)); EIMS, m/z 356 (M^+), 338 (M – H_2O), 297 (M – CO_2Me), 279 (297 – H_2O), 268 (297 –Et), 267 (base peak). Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.91; N, 7.86. Found: C, 70.88; H, 8.06; N, 7.79.

This compound was identical (^{1}H NMR, UV, EIMS, TLC) with that obtained under similar conditions by hydrogenolysis of isoxazolidine **8b**.

Diazotization of 20b (X = NH_2) to 18 and 21. A solution of **20b** (X = NH_2) (73 mg, 0.19 mmol) in dry THF (20 mL) was stirred with *tert*-butyl nitrite (35 μL, 0.30 mmol) in the presence of AcOH (1 μL) until the starting material had disappeared (10 min). TLC (D) indicated the presence of two main products besides two other compounds in smaller amounts. The reaction mixture was evaporated in vacuo at low temperature, the brownish residue was dissolved in CH_2Cl_2 (15 mL), washed with saturated $NaHCO_3$ solution (10 mL, 2 times), and dried ($MgSO_4$), and the organic layer was evaporated. Compounds **18** (28 mg, 43%) and

21 (26 mg, 39%) were separated by preparative TLC (C) and identified by comparison with authentic materials, prepared according to Le Men et al.[25]

X-ray Structure Determination. (A) Zwitterion 7a Hydrate (XR-1). Single crystals of XR-1 suitable for X-ray diffraction study were grown from a saturated MeOH solution. The structure was solved by direct methods (program MULTAN 80): the "best" *E*-map allowed to localize 27 heavy atoms over a total of 32. The remaining heavy atoms were located on a difference Fourier map. After isotropic refinement of heavy atoms, all hydrogen atoms, with the exclusion of those of solvation water, were located on a difference Fourier map. Hydrogen atoms at C(18) and C(25) gave problems in the least-squares process and then were fixed in calculated positions. Hydrogen atoms of solvated water were not clearly localized, because of the high thermal internal motion or the disorder of the same.

(B) Isoxazolidine 8b Hydrobromide (XR-2). Single crystals of XR-2 were obtained by diffusion of diisopropyl ether into a saturated MeOH solution of **8b**·HBr. The structure was solved by locating on a Patterson map the bromine and one oxygen atom. Subsequent cycles of structure-factor calculation and difference Fourier map gave all heavy atoms. After isotropic refinement of heavy atoms, most of the hydrogen atoms became visible on a difference Fourier map, but some of these did not converge and were fixed in calculated positions.

(C) Azepine 9a Dihydrobromide as Methanol Solvate (XR-3). Suitable crystals of XR-3 were obtained by diffusing diisopropyl ether into saturated MeOH solution of **9a**·2HBr. These crystals rapidly lose MeOH with consequent destruction of crystal structure, and the sample used for data collection was sealed in a thin-walled glass capillary. Due to the relatively large linear absorption coefficient and the irregularity of crystal shape, an empirical absorption correction was applied according to Walker and Stuart.[30] The structure was solved by locating bromine atoms on a Patterson map. The atoms were obtained from structure-factor calculations and difference Fourier syntheses. Four broad peaks were successively interpreted and refined as disordered MeOH: the identification of the atoms of the solvate was based on packing contacts. Hydrogen atoms at C(23) were put in calculated position and not refined. Hydrogen atoms of disordered MeOH and one of the two formally deriving from HBr were not clearly identified while the second hydrogen atom of HBr was clearly bonded to N(4).

Tables of observed and calculated structure amplitudes and anisotropic thermal parameters are available on request (T.P.).

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Supplementary Material Available: Tables of fractional coordinates, U_{eq}, bond distances, selected bond and torsion angles, ring-puckering coordinates, asymmetry parameters, and conformation of the non-benzene rings for XR-1, XR-2, and XR-3 (10 pages). Ordering information is given on any current masthead page.

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The Capsaicinoids: Their Separation, Synthesis, and Mutagenicity

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Capsaicin (**1b**), the pungent ingredient in many varieties of *Capsicums* has recently been implicated as a possible carcinogen. When obtained from natural sources **1b** is always accompanied by a number of related homologues. We have isolated seven of these homologues, characterized them, and synthesized them by a general and unique route developed in our laboratories. Also reported are mutagenicity data for **1b** and an extract of red pepper as measured by the Ames assay and the V-79 mammalian cell assay.

Capsaicin **1b**, the principal pungent component in many *Capsicums* (e.g., hot peppers and hot pepper derived substances) has been the target of numerous investigations since it was first isolated 1876.[1] The structure of capsaicin was first determined by Nelson[2] in 1919. The capsaicinoids **1** and dihydrocapsaicinoids **2** have been studied to determine the source of their pungency,[3] their ability to induce sneezing and skin irritation,[4] and with regard to being inhibitors and promoters of substance P.[5] Preliminary data from our laboratories indicate that the capsaicinoids are mutagens,[6] a finding that has been recently confirmed by Nagabhushan and Bhide.[7]

The aforementioned mutagenesis studies were conducted with mixtures of **1** and **2** since natural extracts of hot peppers were used. We were interested in determining if the mutagenic species in these extracts was capsaicin (**1b**) itself, a homologue of capsaicin or dihydrocapsaicin, or some combination of **1** and **2**, hence we needed to be able to prepare each of them. In addition, we required samples of each of the homologues of **1** and **2** for studies dealing with the identification and quantification of capsaicin and its homologues in hot pepper extracts. While a procedure for the preparation of **1b** had been published[8] this method

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