The Structure of Nitronitrosoaconitinic Acid, the Oxidation Product of Aconitine with Nitric Acid[1)]

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The structure of nitronitrosoaconitinic acid, which is an oxidation product of aconitine with nitric acid, was confirmed by spectroscopic methods. This compound is a new type of acid, a 2-nitro-2-cyclohexen-1-one, with a pK_a value comparable to that of carboxylic acids.

A study of the oxidation of aconitine (**1**) with nitric acid was first performed by Brady.[2,3a)] The oxidation of aconitine (**1**), mesaconitine (**2**), and their derivatives has been found to yield a nitro *N*-nitroso derivative (**3**).[2,3b,4-7)] Compound **3** has been called nitronitrosoaconitinic acid by Suginome.[4)] Two alternative molecular formulae, $C_{31}H_{33}O_{13}N_3$[4)] and $C_{31}H_{35}O_{13}N_3$,[5,7)] have been proposed for **3** on the basis of the elemental analysis. Moreover, Suginome found that, although it retained both benzoyl and acetyl groups of **1**, one of the four methoxyl groups and alkyl group attached to the tertiary nitrogen of **1** are lost in the transformation.[4)] Subsequently, extensive studies[5-7)] have been carried out, but the structure still remains unknown. Compound **3** has now been subjected to modern structural analyses; in this paper, we wish to propose the **3** structure for this compound.

By field desorption mass spectrometry, **3** was confirmed to have a molecular formula of $C_{31}H_{35}O_{13}N_3$ [*m/e* 658 $(M+1)^+$]. The infrared spectrum exhibited bands attributable to hydroxyl groups at 3400—3700; to an α,β-unsaturated carbonyl and acyl carbonyl groups, at 1700 and 1720; to a nitro group, at 1373 and 1538, and to a benzene ring C–H bond, at 720 cm^{-1}. The UV spectrum in ethanol showed strong absorption bands at 230 (log ε; 4.2) and 343 nm (log ε; 3.4) attributable to an α,β-unsaturated carbonyl group. The ^1H NMR spectrum ($CDCl_3$, δ) showed the presence of an acetoxyl group (1.40, s, 3H), three methoxyl groups (3.18, 3.33, and 3.82, each 3H), and a benzoyloxy group and an olefinic proton (7.26—8.10, m, 6H). The removal of the two acyl groups by the alkaline hydrolysis of **3** afforded a compound (**4**), $C_{22}H_{29}O_{11}N_3$, designated as nitronitrosoaconinic acid,[4)] [*m/e* 512 $(M+1)^+$]. The infrared spectrum exhibited bands attributable to hydroxyl groups at 3300—3700; to an α,β-unsaturated carbonyl group, at 1700, and to a nitro group at 1370 and 1535 cm^{-1}. The UV spectrum in ethanol showed strong bands at 230 (log ε; 4.1) and 343 nm (log ε; 3.1) attributable to an α,β-unsaturated carbonyl group. The ^1H NMR spectrum (D_2O, δ) revealed the presence of three methoxyl groups (3.30, 3.34, and 3.57, each 3H) and an olefinic proton (7.63, s, 1H) and indicated the generation of an olefinic proton in the transformation of **1** into **3**. On the acetylation of **4** with acetyl chloride, all of the four free hydroxyl and *N*-nitroso groups reacted and *N,O*-pentaacetyl derivative (**5**), dp 275—279 °C, was obtained as in the case of the acetylation of **3**.[4)] By chemical-ionization mass spectrometry, **5** was confirmed to have the molecular formula of $C_{32}H_{40}O_{15}N_2$ [*m/e* 710 $(M+NH_4)^+$]. The infrared spectrum exhibited bands attributable to an α,β-unsaturated carbonyl and acyl carbonyl groups at 1695 and 1740, and to a nitro group at 1362 and 1533 cm^{-1}. The UV spectrum in ethanol showed strong bands at 228 (logε; 4.3) (shoulder) and 343 nm (logε; 3.8) attributable to an α,β-unsaturated carbonyl group. The ^1H NMR spectrum ($CDCl_3$, δ) established the presence of four acetoxyl and an *N*-acetyl groups (2.00, 2.03, 2.09, 2.11, and 2.20, each 3H), three methoxyl groups (3.32, 3.36, and 3.40, each 3H), and an olefinic proton (7.18, s).

The strucutre of **3** was deduced by the correlation of the ^{13}C NMR signals of **1** with those of **3**. The reported[8)] ^{13}C signal assignments of **1**, together with those of **3**, **4**, and **5**, are shown in Table 1. Assignments of the resonances to individual carbon atoms were achieved by using the ^1H single-frequency off-resonance decoupling technique, the chemical shift theory, and a comparison of their chemical shifts with those of aconitine type diterpenoid alkaloids.[8)] A comparison of the ^{13}C signal assignments of **1** and **3** reveals the presence of the carbonyl group (singlet at 186.7 ppm) and the lack of C-3 carbon resonance at 70.4 ppm (doublet) in **3**. Thus, the carbonyl group was formed by the oxidation of the secondary hydroxyl group in Ring A. The low-field resonances at 142.8 and 148.8 ppm in **3** are assigned to the olefinic carbons, which correspond to a methine and a quarternaly carbon with a nitro group. The position of this double bond can be assigned to C-1 and C-2 on the basis of the observation of the UV spectrum of **3** and the lack of C-1 and C-2 carbon resonances at 83.4 and 36.0 ppm in **1**. The observation of the unexpected downfield shift (3.3 ppm) of the C-12 carbon in **3** relative to **1** suggests that it is preferable to interchange the chemical shifts of the C-2 and C-12 carbons in **1**.[8)] By this interchange, this downfield shift is diminished to 1.3 ppm. On the other hand, on the basis of the observation of the spin-lattice relaxation-time (T_1) maesurements (C-17; 0.2 s and C-16; 0.6 s), the reported assignments of the chemical shifts of C-17 (61.0) and C-16′ (60.7)[8)] have been interchanged.[1)] The separation of the closely spaced peaks of C-17/C-16′ into positive and negative signals was observed. These data suggest that a large structural change took place only in the A-ring, more specifically, that

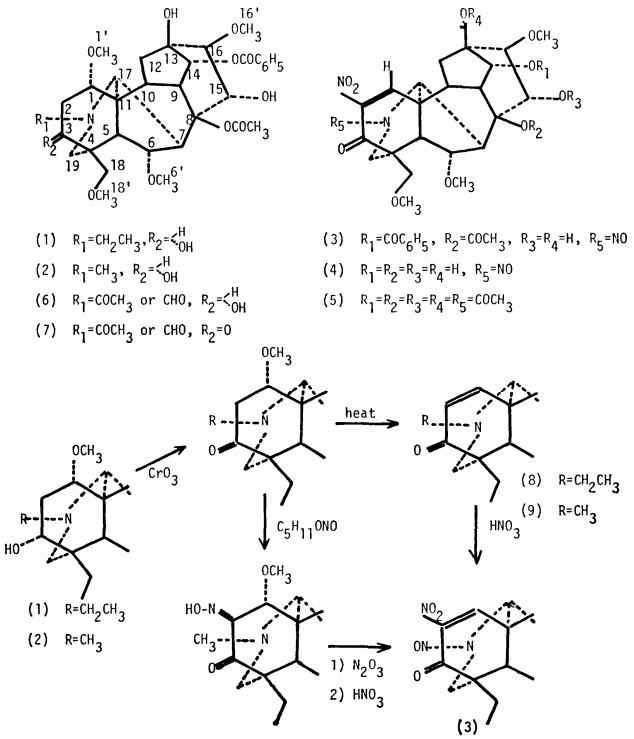
Table 1. ^{13}C chemical shifts of aconitine (**1**),[a] nitronitrosoaconitinic acid (**3**), nitronitrosoaconinic acid (**4**), and nitro-*N*-acetylaconinic acid tetraacetate (**5**)

Carbon	**1**[a,b]	**3**[b]	**4**[c]	**5**
1	83.4 (d)	142.8 (d)	145.8 (d)	144.1 (d)
2	34.0[g] (t)	148.8 (s)	150.7 (s)	149.1 (s)
3	70.4 (d)	186.7 (s)	189.0 (s)	188.4 (s)
4	43.2 (s)	48.5[d] (s)	50.4[d] (s)	49.0[d] (s)
5	46.6 (d)	51.1 (d)	55.4 (d)	50.3 (d)
6	82.3 (d)	81.1 (d)	82.5 (d)	82.0 (d)
7	44.8[d] (d)	48.0 (d)	49.2 (d)	47.9 (d)
8	92.0 (s)	89.5 (s)	78.7 (s)	87.9 (s)
9	44.2[d] (d)	42.1 (d)	48.1 (d)	41.9 (d)
10	40.8 (d)	36.7 (d)	38.6 (d)	37.2 (d)
11	49.8 (s)	50.5[d] (s)	51.7[d] (s)	51.7[d] (s)
12	36.0[g] (t)	37.3 (t)	39.7 (t)	37.9 (t)
13	74.0 (s)	74.1 (s)	77.6 (s)	79.9 (s)
14	78.9 (d)	78.3[e] (d)	80.0[e] (d)	76.1[e] (d)
15	78.9 (d)	78.5[e] (d)	81.5[e] (d)	77.6[e] (d)
16	90.1 (d)	89.9 (d)	92.9 (d)	88.2 (d)
17	60.7[f] (d)	62.6 (d)	64.8 (d)	52.5 (d)
18	75.6 (t)	70.5 (t)	72.2 (t)	70.1 (t)
19	48.8 (t)	42.7 (t)	44.0 (t)	46.6 (t)
N-CH_2	46.9 (t)	—	—	—
CH_3	13.3 (q)	—	—	—
1′	55.7 (q)	—	—	—
6′	57.9 (q)	58.3 (q)	58.4 (q)	59.1 (q)
16′	61.0[f] (q)	61.4 (q)	61.9 (q)	61.6 (q)
18′	58.9 (q)	59.2 (q)	59.5 (q)	59.1 (q)
C=O	172.2 (s)	172.0 (s)	—	C=O 168.2×2 (s)
CH_3	21.3 (q)	21.2 (q)	—	170.1×2 (s)
C=O	165.9 (s)	165.7 (s)	—	170.3 (s)
C_6H_5	129.8 (s)	129.2 (s)	—	CH_3 20.9 (q)
	129.6 (d)	129.6 (d)	—	21.9 (q)
	128.6 (d)	128.8 (d)	—	21.2×2 (q)
	133.2 (d)	133.7 (d)	—	21.6 (q)
				22.0 (q)

a) Results reported by Pelletier and Djarmati.[8] b) δ, ppm downfield from TMS in $CDCl_3$. c) δ, ppm downfield from TMS in CD_3OD. d) and e) These two values of each compound may be interchanged. f) and g) The reported assignments[8] of the chemical shifts of C-17 (61.0) and C-16′ (60.7) have been interchanged[1]; those of C-2 (36.0) and C-12 (34.0) are also interchanged.

the α,β-unsaturated carbonyl group was introduced into the A ring. The nitro group could be attached to either the α (C-2) or β (C-1) position of the α,β-unsaturated carbonyl group. Compound **3** can be obtained[6] from **2** *via* chromium trioxide oxidation to a ketone, followed by nitrosation at the carbon α to the carbonyl and oxidation with nitric acid. This sequence of reactions allows us to confirm that the nitro group is definitely attached to the C-2. Contrary to the significant differences observed in the chemical shifts of the C-1, C-2, and C-3 between **1** and **3**, only slight differences for C-4, C-5, C-7, C-10, C-18, and C-19 can be seen. These slight differences can be explained as results of the β and γ effects accompanying changes in the C-1, C-2, and C-3 carbons and the amino nitrogen in **1**. On the basis of all the results described above, the structure of nitronitrosoaconitinic acid is formulated as **3**; the series of reactions from **1** and **2** to afford **3**[6] are depicted in Scheme 1. According to this reaction process, the formation of **3** from oxonitine (**6**),[9,10] oxoaconitine (**7**),[5,7] and demethanolaconitinone (aconitoline) (**8**)[5-7] can be understood readily. These experimental data[6] suggest that Compound **9** is not demethanolanhydromesaconitinone $C_{32}H_{37}O_9N$,[6] but demethanolmesaconitinone $C_{32}H_{39}O_{10}N$, as is shown by the partial structure in Scheme 1. The chemical shifts of all of the carbons in **4** and **5** shown in Table 1 are in agreement with the assigned structures, **4** and **5**, considering the effects of using different solvents and the substitution of the hydroxyl for the acetoxyl groups.

The pK_a value of **3** was determined to be 4.9 by spectrophotometric measurements at a wavelength of 343 nm (λ max)[11] in buffer solutions. This value suggests that the acid strength of **3** is nearly equal to that of carboxylic acids. In the ^1H NMR spectrum, a signal due to an olefinic proton at δ 7.63 of **4** in D_2O disappeared slowly in the presence of a small



Scheme 1.

amount of alkali. Therefore, a system formed by the introduction of a nitro group into the α carbon of the double bond of the α,β-unsaturated carbonyl group is considered to be responsible for the acidity. No study concerning the acidity of such a structure has been reported, and further studies are presently in progress in our laboratories.

Experimental

The mps are uncorrected. The IR spectra were determined in KBr discs. The UV spectra were measured by means of a Shimadzu UV-300 spectrophotometer. The MS were measured by JEOL JMS-D300 and Shimadzu GCMS-9000 spectrometers. The Carbon-13 FT NMR spectra were taken at 25.00 MHz using a JEOL FX-100 spectrometer equipped with a JEC 980-24 K computer and 5 mm o. d. tubes. The FT-measurement conditions were: spectra width, 5 kHz; pulse width, 5 μs (45°); pulse repetition time, 1.0 s; number of data points, 8192; numbers of transients, 5000—40000. The values of pH were determined using a Corning model 7 pH meter.

Nitronitrosoaconitinic Acid (3). Mesaconitine (**2**) (2.3 g) was oxidized in 6 ml of HNO_3 (d 1.42) according to the method of a previous report[4] by heating in a water bath at 80 °C for 40 min. The precipitate (2.1 g) formed on the dilution of the reaction mixture was placed in a column of silica gel (100 g) and eluted with chloroform–methanol (100:1). This yielded **3** (575 mg) (needles of pale yellow) after recrystallization from acetone–water; dp 230 °C. UV: λ_{max}^{EtOH} 230 nm (log ε; 4.2) and 343 (log ε; 3.4); IR: ν_{max}^{KBr} 3700—3400, 1720, 1700, 1538, 1373, 720 cm^{-1}; NMR ($CDCl_3$, δ): 1.40 (3H, s, $OCOCH_3$), 3.18 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 7.26—8.10 (6H, m, an olefinic and aromatic protons); MS: m/e 658 $(M+1)^+$. Found: C, 55.71; H, 5.09; N, 6.08%. Calcd for $C_{31}H_{35}O_{13}N_3\cdot 1/2H_2O$: C, 55.85; H, 5.44; N, 6.30%.

Nitronitrosoaconinic Acid (4). Nitronitrosoaconitinic acid (**3**) was hydrolyzed according to previous report[4] by boiling it with 1 M barium hydroxide solution for 1 h. After cooling, the solution was passed through a column of an ion-exchange resin (Dowex-50W). The eluate was then evaporated. The residue was placed in a column of silica gel and eluted with ethanol. Recrystallization from water gave crystals: **4**; dp 287 °C. UV: λ_{max}^{EtOH} 230 nm (log ε; 4.1) and 343 (log ε; 3.1); IR: ν_{max}^{KBr} 3700—3300, 1700, 1535, 1370 cm^{-1}; ^1H NMR (D_2O, δ): 3.30 (3H, s, OCH_3), 3.34 (3H, s, OCH_3), 3.57 (3H, s, OCH_3) and 7.63 (1H, olefinic proton); MS: m/e 512 $(M+1)^+$. Found: C, 49.43; H, 5.60; N, 7.54%. Calcd for $C_{22}H_{29}O_{11}N_3\cdot H_2O$: C, 49.90; H, 5.90; N, 7.93%.

Nitro-N-acetylaconinic Acid Tetraacetate (5). A mixture of nitronitrosoaconinic acid (**4**) (100 mg) and acetyl chloride (2 ml) was allowed to stand in a sealed tube for a week. After the subsequent removal of the acetyl chloride, methanol (1 ml) was added to the residue, and the resulting solution was added to ice-cold water. The precipitate (97 mg) thus formed was purified on a silica-gel column by eluting with chloroform–methanol; from the eluate, crystals (34 mg) were obtained; dp 275—279 °C. UV: λ_{max}^{EtOH} 228 nm (log ε; 4.3) (shoulder) and 343 (log ε; 3.8); IR: ν_{max}^{KBr} 1740, 1695, 1533 and 1362 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 2.00, 2.03, 2.09, 2.11 and 2.20 (3H, singlet each, $OCOCH_3\times 4$ and $NCOCH_3$), 3.32, 3.36 and 3.40 (3H, singlet each, $OCH_3\times 3$) and 7.18 (1H, s, an olefinic proton); MS: m/e 710 $(M+NH_4)^+$. Found: C, 54.36; H, 5.60; N, 4.40%. Calcd for $C_{32}H_{40}O_{15}N_2\cdot H_2O$: C, 54.08; H, 5.96; N, 3.94%.

Spectrophotometric Determination of the pK_a of Nitronitrosoacnitinic Acid (3). To a series of 5-ml portions of a saturated solution of **3** in water, 5-ml portions of 0.2 M buffers of different pH values were added, and then the resultant pH values of the mixtures were determined. A series of spectra were recorded at 22 °C. The pK_a value was determined at 343 nm according to the method described by Albert and Serjeant.[12] For the pH range of 4.1—5.6, acetate buffers were employed, and for that of 5.8—6.2, phosphate buffers. The spectra of the molecular and ionized species were determined in 0.005 M HCl and 0.005 M NaOH respectively.

Spectrometric Identification of the Olefinic Proton. To nitronitrosoaconinic acid (**4**) (30 mg) dissolved in 0.2 ml of D_2O, 0.05 ml of a solution of NaOD (60 mg) in D_2O (15.2 ml), divided into three protions, was added over a one-hour period at 22 °C. The NMR spectra were obtained during this period.

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