ON THE PROTONATION SITE OF RAUWOLFIA ALKALOIDS IN HIGHLY CONCENTRATED SULPHURIC ACID SOLUTIONS

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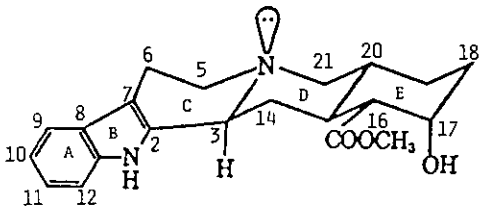
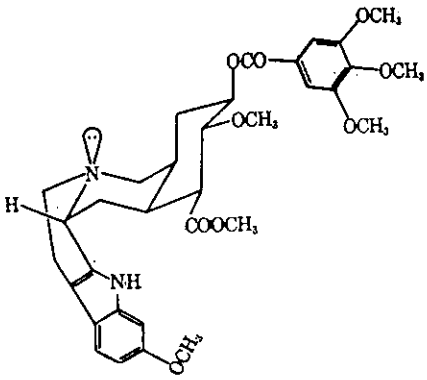
Abstract — ^{13}C-Nmr spectroscopy has been used to investigate the protonation site of the Rauwolfia alkaloids yohimbine, reserpine and reserpiline in highly concentrated sulphuric acid solutions (≈18M H_2SO_4). The chemical shifts which occur upon protonation reveal unambiguously that the alkaloids protonate on C-7, the β-position with respect to their indole ring, and suggest that the indoleninium cations of the methoxy-substituted alkaloids have principally an enamine structure.

In a recent investigation on protonation equilibria of some Rauwolfia alkaloids in highly concentrated sulphuric acid solutions,[1] it was found that their ionizations obeyed the H_I acidity function established by Hinman and Lang[2] for indole ring protonation, but they were considerably weaker bases than alkylindoles. This was imputed to the strong electrostatic destabilizing effect of the positive charge on the nonindole nitrogen atom present in their tetrahydro-β-carboline skeleton, which is easily protonated in aqueous media.[3] On the basis of such behaviour and the characteristics of the uv-vis spectra of the protonated species, it was suggested that, as in typical indoles, protonation took place on carbon yielding indoleninium cations.[4] Although these equilibria do not seem to take place in biological processes, its study affords valuable information of theoretical interest in connection with other heterocyclic systems and of practical importance to many aspects of the chemistry of these compounds.[5]

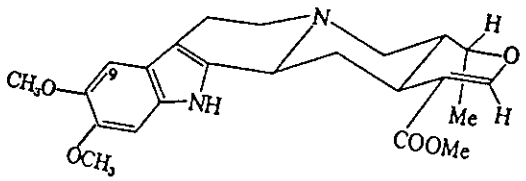
This paper describes the results of an investigation of the ^{13}C-nmr spectra of some Rauwolfia alkaloids in highly concentrated sulphuric acid solutions. It was undertaken to definitively establish the protonation site and to examine the influence of the methoxy substituents on the structural characteristics of the protonated cations. For this purpose, we have chosen the Rauwolfia alkaloids yohimbine (1), reserpine (2), and reserpiline (3), which serve as models of different patterns of methoxy substitution.



(1) Yohimbine



(2) Reserpine



(3) Reserpiline

EXPERIMENTAL

Yohimbine hydrochloride, reserpine, and reserpiline hydrochloride were generously donated by Boehringer and Sohn, and 2,3-dimethylindole, 1,3,3-trimethyl-2-methylene indoline, and 2,3,3-trimethylindolenine were purchased from Sigma. All these materials were of the best available commercial quality and were used without further purification. Tetrahydroharmine (6-methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline) and reserpic acid were prepared by known procedures.[6,7] Sulphuric acid (Merck) and deuterated sulphuric acid (Sigma) were used to prepare solutions of the protonated compounds under study.

Fourier transform (FT) ^{13}C-nmr spectra were run at 50.2 MHz on a Varian XL-200 spectrometer. Depending on sample concentration and solvent, averaging of between 10^3 and 10^5 transients was used for completely proton decoupled spectra and about three times as many for attached proton test (APT) and single-frequency off-resonance decoupling spectra (SFORD).

The spectra of the free bases were recorded in $CDCl_3$ or DMSO-d_6 as the solvent, the internal lock and the internal standard. For the protonated species, ∿18M H_2SO_4 and benzene were used as the solvent and the external standard (coaxial tubes), respectively. Chemical shifts are expressed in δ units in ppm downfield relative to tetramethylsilane.

RESULTS AND DISCUSSION

The ^{13}C-nmr chemical shifts of yohimbine hydrochloride (1·HCl), reserpine (2), and reserpiline hydrochloride (3·HCl), and their respective cations (1p, 2p, 3p) are given in Table I. Since the alkaloids have pK_a values for indole-ring protonation ranged between -7 and -9 on the H_I acidity scale,[1] the spectra of the protonated cations were taken in ∿18M (96% w/w) sulphuric acid solutions in order to ensure

complete protonation. Assignments of the ^{13}C resonances were aided by ^{13}C-nmr spectra of well-documented reference compounds,[9-13] the APT techniques, and the SFORD spectra.

The chemical shifts of reserpine recorded in Table I are entirely similar to those previously reported by Wenkert et al,[8] and, therefore, our assignments were made on this basis. The assignments of the chemical shifts of yohimbine hydrochloride were aided by those of the free base reported by the same authors.[8,10] The ^{13}C-nmr spectra of reserpiline were not found in the bibliography, therefore the assignments of the chemical shifts of reserpiline hydrochloride were made with the aid of the ^{13}C-nmr data on other ajmalicinoid alkaloids[9] and averaging the methoxy substitution effects on the benzene ring reported for other indole compounds similarly substituted.[12]

Since no literature data were available on the effect of protonation of the indole ring on the ^{13}C-nmr resonances, we have also studied the ^{13}C-nmr spectra of 2,3-dimethyl indole, 1,3,3-trimethyl-2-methylindoline, 2,3,3-trimethylindolenine, and their respective cations in concentrated sulphuric acid solutions (Scheme I). It is well known from uv-vis, ir, and ^{1}H-nmr spectroscopies that simple indoles protonate preferently on the β-carbon yielding indoleninium cations.[4]

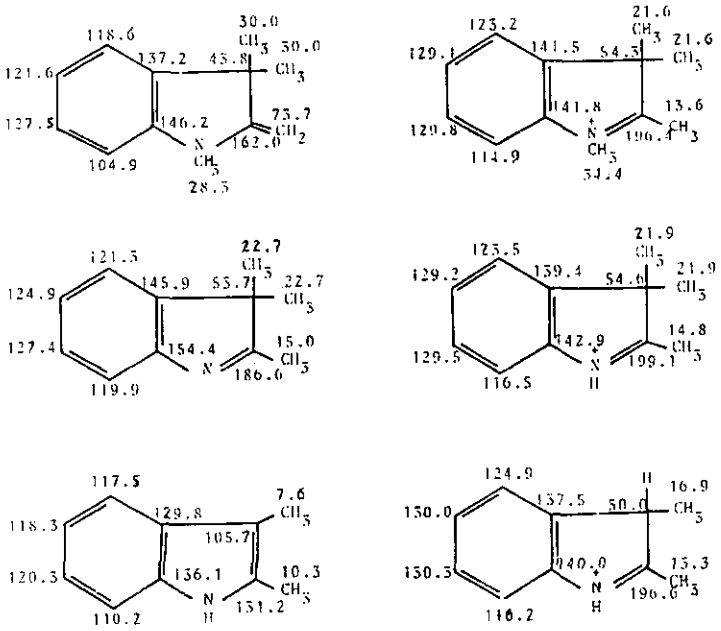
TABLE I: Carbon shifts of yohimbine (1), reserpine (2) and reserpiline (3), and their respective cations (1p, 2p, and 3p).

Carbon	1	1p	2[d]	2p[a]	3	3p[b]
2	129.5	182.2	130.2	179.6	127.0	181.2
3	57.5	61.9	53.6		70.8	
5	52.0	54.0	51.1	51.7	51.8	52.4
6	18.8	20.7	16.7	14.7	18.8	24.3
7	105.7	50.1	107.7	58.6	103.6	52.3
8	126.1	134.5	121.9		118.8	
9	118.3	125.0	118.2		100.8	
10	119.4	130.9	108.7		144.8	148.5
11	122.0	132.2	155.8	152.1	146.8	150.7
12	111.8	117.8	95.0	83.6	95.7	c
13	136.7	140.3	136.1		131.2	
14	31.2	27.3	24.1	24.1	27.7	27.7
15	34.2	32.8	33.8	29.0	24.1	22.5
16	51.7	49.7	51.6	47.2	106.2	96.7
17	66.4	82.1	77.7	78.6	154.7	172.2
18	32.2	28.3	77.7	67.6	--	--
19	22.3	25.6	29.6	28.0	73.1	77.7
20	37.2	36.7	32.2	30.6	34.0	34.3
21	60.4	59.8	48.9	48.6	54.2	54.2
22	172.5	176.9	172.5	173.3	166.7	179.0

[a] Unassigned signals (see the text): 116.2(s), 123.6(s), 124.6(s), 127.7(d), and 136.1(s)
[b] Unassigned signals (see the text): 105.0(d), 119.9(s), 126.5(s), and 134.5(s)
[c] Unobserved signal
[d] From reference 24

As can be seen from the data in Scheme I, protonation of 2,3-dimethylindole is accompanied by significant and diagnostically useful shifts which allow to analize the spectra of the indoleninium moiety in the more complex molecules of the alka-

loids. In particular, the resonance of C-2 is considerably deshielded ($\Delta\delta \sim 60$ ppm), whereas that of C-3, the protonation site, is shielded about 50 ppm. Moreover, this latter signal, which appears as a doublet in the non-decoupled spectra obtained in H_2SO_4, changes to a singlet in D_2SO_4. Deuterium exchange experiments have demonstrated that in strong sulphuric acid solutions the indoleninium cations interchange the deuterium atoms preferentially at N and C-3.[14]



SCHEME I

The general procedure for the assignment of the carbon resonances of the protonated alkaloids will be described with yohimbine. The peaks of the ^{13}C-nmr of yohimbine in 18M H_2SO_4 have been separated into two groups; those corresponding to unsaturated carbons at lower field (δ 200-100 ppm) and those corresponding to saturated carbons at higher field (δ<100 ppm). In the low-field portion most of the resonances are very similar to those of the 2,3-dimethylindoleninium cation and, therefore, their assignments have been made on this basis.

The high-field portion of the spectra is similar to that of unprotonated yohimbine hydrochloride, which allows assignments of all the carbons in the C, D, and E rings of yohimbine skeleton, except for C-7, and C-17. The resonance of C-7 was assigned without ambiguity at δ 50.1 ppm by comparison with the resonance of this carbon in 2,3-dimethylindoleninium cation and the change of multiplicity in the nondecoupled spectra obtained in H_2SO_4 and D_2SO_4. The C-17 resonance was assigned to 82.1 ppm; owing to the protonation of the hydroxy group, this C-resonance should shift to downfield.

Most of the signals of the spectra of reserpine and reserpiline in concentrated sulphuric acid solutions were assigned in a similar way. However, the existence of

further reactions after protonation made very difficult to assign without ambiguity some of the signals of these spectra. Unfortunately, it was not possible to obtain the spectra at initial times of protonation due to the prolonged times required for the obtainment of significant off-noise spectra of these complex molecules. On the other hand, the use of simplest tetrahydro-β-carbolines as model compounds neither helped to the elucidation of these spectra due to the propensity of these compounds to polimerize upon protonation.

The analysis of the ^{13}C-nmr spectra of reserpine in ∿18M H_2SO_4 was complicated by decomposition reactions previously observed in the pK_a measurements of this alkaloid. Thus, these spectra contain more signals than carbon atoms in the molecule of reserpine. Decomposition of reserpine through hydrolysis and oxidative reactions in moderately concentrated acidic media has been well documented.[15,16] The hydrolysis product, trimethoxybenzoic acid, also suffers protonation and subsequent decarboxylation reactions.[17] In fact, the spectra of reserpine in ∿18M H_2SO_4 acid contained all the peaks observed in the ^{13}C-nmr spectrum of trimethoxybenzoic acid obtained under the same acidic conditions. Then, these peaks were substracted and the reserpine spectrum reduced to 24 remaining signals. Similar signals were also observed in the ^{13}C-nmr spectrum of reserpic acid obtained in ∿18M H_2SO_4. The identification of most of them was straightforward following the above described procedure and by comparison with the tetrahydroharmine spectrum obtained at initial times. However, difficulties appear for the assignment of the signals at 116.2 (s), 123.6 (s), 124.6 (s), 127.7 (d), and 136.1 (s) to the remaining unassigned C-3, C-8, C-9, C-10, and C-13 atoms without considering the possibility of aromatization or sulphonation reactions on the tetrahydro-β-carboline moiety of the reserpine skeleton.[18]

However, in spite of the fact that reserpine oxidizes in acidic media to 3,4-dehydroreserpine and tetradehydroreserpine,[15] the presence of such compounds has not been detected in ∿18M sulphuric acid solutions of reserpine upon a 10-12 hours standing necessary to obtain the ^{13}C-nmr spectra of this compound (3,4-dehydroreserpine and tetradehydroreserpine possess unambiguous uv-vis, fluorescence, and ^{13}C-nmr spectra).[18,19]

On the other hand, the fact that sulphonation on the benzene nucleus of reserpine took place in highly concentrated sulphuric acid solutions was inferred from tetrahydroharmine. This compound, which possesses the basic structural unit of the tetrahydro-β-carboline nucleus of reserpine, renders upon solution in highly concentrated sulphuric acid a C-10 substituted derivative. This carbon resonates at 120.2 ppm.[20] Therefore, some of the singlets at 116.2, 123.6 or 124.6 ppm. should be assigned to this carbon in protonated reserpine. Nevertheless, the assignment of the remaining signals would require the quaternization of C-3 or C-9 methine carbons of reserpine to account for the existence of the only remaining doublet at 127.7 ppm. However, the possibility of a double sulphonation seems very improbable under the experimental conditions used to obtain the spectra.[21]

At this point, it is interesting to consider the previously suggested hypothesis that the bathochromic shifts experienced by the uv-vis spectra of the alkaloids

upon protonation, seem to involve the longer conjugated system of α-methylenindoline extending over the carbon rings in the protonated molecules.[1] On this basis and aided by the literature data on alkaloids having a such chromophore,[12] the peaks at 127.7 (d), 136.1 (s), and 116.2 (s) might be tentatively assigned to C-9, C-13 and C-3 atoms, respectively. In a similar way, the unassigned signals in the spectra of protonated reserpiline at 105.0 (d), 119.9 (s), 126.5 (s), and 134.5 (s) might be assigned to C-9, C-3, C-8, and C-13, respectively.

The apparent existence of the indoleninium cations of the methoxy-substituted alkaloids reserpine and reserpiline in the enamino form is a point of great interest in relation to the Rauwolfia alkaloid chemistry. Thus, the more characteristic reactions of these compounds[5] have been rationalized by reaction mechanisms that almost invariably involve the formation of indolenines and tautomeric equilibria of their imino and enamino forms. Most of these reactions, such as epimerizations at C-3,[22,23] are known not to depend upon steric factors but rather upon electrophilic factors associated with the substituents in the benzene ring of the alkaloids. Thus, deserpidine, the non-methoxylated derivative of reserpine, epimerizes much more slowly than reserpine.

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