Carbon-13 NMR parameters of orellanine

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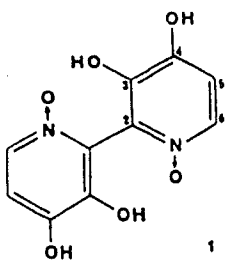
RESUMEN.—Las señales del espectro de ^{13}C RMN de la orellanina han sido atribuidas definitivamente con la ayuda de los espectros de correlación ^{1}H-^{13}C y de las constantes de acoplamiento a larga distancia C-H.

SUMMARY.—The signals of the ^{13}C NMR spectrum of orellanine have been unambiguously assigned with the help of ^{1}H-^{13}C correlation spectra and long-range C-H coupling constants.

INTRODUCTION

The structure of orellanine, the presence of which in *Cortinarius orellanus* (Fries) and *Cortinarius speciosissimus* (Kühn. and Romagn.) seems to be responsible for the toxicity of these species (1-5) was suggested, for the first time, in 1979 (6). Its structure was definitely established after a total synthesis (7,8) of 3,3',4,4'-tetrahydroxy 2,2'-bipyridine N,N-dioxide, **1**, showed it to be identical to orellanine.



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Nevertheless, there rest some discrepancies concerning the ^{13}C-NMR spectrum of orellanine. Antkowiak and Gessner (6) published the chemical shifts of four carbons in tetrafluoroacetic acid (TFA), Tiecco *et al.* (8) all the chemical shifts in DMSO-d_6, and Prast *et al.* (4) in NaOD/D_2O. The two former groups made no attribution, and the latter differentiates the C-H carbons only.

On the other hand, Richard (9) gave an assignment for the ^{13}C spectrum of orellanine in DMSO-d_6, but his results, for the 3 substituted carbons, were not in agreement with those of Dehmlow and Schulz (10).

RESULTS AND DISCUSSION

The standard ^{13}C spectrum of orellanine in DMSO-d_6 exhibits five signals at 110.08, 130.14, 131.88, 150.43 and 155.01 ppm. Using the gated decoupling technique, the signals at 110.08 and 131.88 ppm appear as two doublets with coupling constants of 169.0 and 192.3 Hz respectively. This observation allows their assignment to C-5 and C-6 respectively (carbons α to the nitrogen atom in pyridine N-oxide (11,12) are known to be those with the largest ^{1}JCH).

It is now possible to assign the two identical doublets in the ^{1}H spectrum with the help of a ^{1}H-^{13}C correlation spectrum based on a JCH of 180 Hz. It is found that the ^{13}C signal at 131.88 ppm (C-6) is correlated with the doublet ($J=$ 6.8 Hz) at 8.260 ppm in the ^{1}H spectrum, and the signal at 110.08 ppm (C-5) with the other proton signal at 7.148 ppm.

The last problem arises in the assignment of the three substituted carbons. First, we attempted to resolve this problem, as in the benzene series (13), by the prediction of the chemical shifts using known empirical additive substituent parameters for the hydroxyl group in the pyridine series (all of those for the pyridine N-oxide are not yet known).

Table 1 shows the chemical shifts of 2-hydroxy pyridi-

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TABLE 1

^{13}C chemical shifts in DMSO-d_6 of pyridine and some of its hydroxylated derivatives. [a,b]

	7	2	3	4	5	6
C-2	149.67	162.3[a]	137.8[a]	139.8[a]	158.32	164.35
C-3	123.87	119.8[a]	153.5[a]	115.9[a]	147.21	98.61
C-4	136.04	140.8[a]	121.4[a]	175.7[a]	115.47	167.52
C-5	123.87	104.8[a]	123.8[a]	115.9[a]	105.40	99.42
C-6	149.67	135.2[a]	140.0[a]	139.8[a]	123.87	135.73

[a]Reference (14).

[b]**2**; 2-hydroxypyridine; **3**: 3-hydroxypyridine; **4**: 4-hydroxypyridine; **5**: 2,3-dihydroxypyridine; **6**: 2,4-dihydroxypyridine; **7**: pyridine.

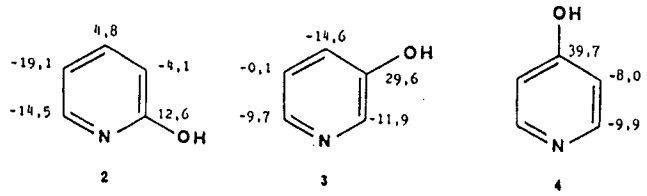


Figure 1

Empirical substituent parameters for the hydroxyl group in the pyridine series

ne, 2 (14), 3-hydroxy pyridine, **3** (14), 4-hydroxy pyridine, **4** (14), 2,3-dihydroxy pyridine, **5**, and 2,4-dihydroxy pyridine, **6**. If compounds **2**, **3** and **4** are compared to pyridine, **7**, in the same solvent, parameters for the hydroxyl group can be obtained (Fig. 1).

It is then possible to compare the calculated and experimental effects in the case of the two dihydroxy pyridines **5** and **6** (Fig. 2).

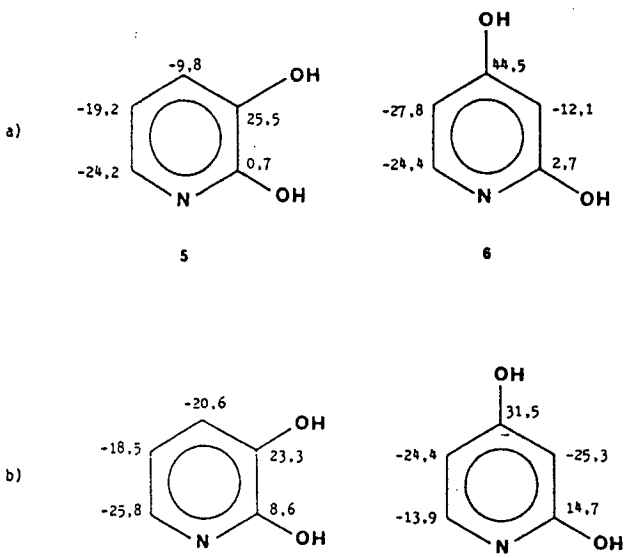


Figure 2

a) Calculated (assuming additivity of substituent parameters) and b) experimental effects of hydroxyl group for two dihydroxy pyridines

It is clear that the calculated values do not allow for the correct prediction of chemical shifts using the experimental parameters of Figure 1. This discrepancy may be explained by the existence in 2-hydroxy- and 4-hydroxy pyridines of a pyridine/pyridone equilibrium, shifted towards the pyridone form in **2** and **4** (14), but perhaps modified by the second hydroxyl group in **5** and **6**.

A similar equilibrium exists in 2-hydroxy- and 4-hydroxy pyridine N-oxides, and it has been recently shown (15) that, in DMSO-d_6, the 2-hydroxy pyridine N-oxide is found in the N-hydroxy 2-pyridone form.

Thus, we decided to tentatively assign the substituted carbon signals of orellanine by the way of long-range *JCH* coupling constants, considering the fact that ^3JCH have been found to be larger than ^2JCH in all the hydroxy pyridine N-oxides studied (15).

In the normal gated decoupling spectrum of orellanine in DMSO-d_6, the signals are too broad to allow the measurement of long-range coupling constants, and a drop of H_2SO_4 0.1 N has to be added in order to obtain sharp signals. The coupling constants are collected in Table 2.

If these values are compared (Fig. 3) to those published for the pyridine N-oxide (12, 16) and for the pyridinium cation (16, 17), the signal at 155.01 ppm which shows two long-range couplings must be assigned to C-4.

The two remaining signals at 130.14 and 150.43 ppm both exhibit only one ^3JCH. A ^1H-^{13}C correlation spectrum based on a *JCH* of 6 Hz indicates that the ^{13}C signal at 130.14 ppm is correlated with the ^1H signal at 8.260 ppm (H-6) and must be C-2. Similarly, the signal at 150.43 ppm is correlated with H-5 at 7.148 ppm and must belong to C-3.

Thus the assignments we propose in Table 2 are confirmed for the first time, and are in agreement with those of Dehmlow and Schulz (10). These results together with the observed solvent effects (11) calculated by the comparison of pyridine N-oxide in DMSO-d_6 and TFA, seem to indicate that the assignment of substituted carbons of orellanine in TFA made by Cohen-Addad *et al.* (18) are not correct and should be: C-2 (127.1), C-3 (148.3) and C-4 (160.2).

Finally, the chemical shift at 155.01 ppm for C-4 of orellanine, if compared with the value 171.9 ppm for C-4 of the dissymmetric compound **8** synthetised by Dehmlow and Schulz (10), shows that orellanine is probably not in the N-hydroxy 4-pyridone form, unlike 2-hydroxy pyridine N-oxide (15).

TABLE 2

^{13}C chemical shifts (ppm) and JCH (Hz) of orellanine in DMSO-d_6 without and with a drop of H_2SO_4 0.1 N

	DMSO-d_6	DMSO-d_6 $+H^+$
C-5	110.08 [^{1}JCH = 169.0]	110.29 [^{1}JCH = 169.3 – ^{2}JCH = 2.7]
C-2	130.14	129.89 [^{3}JCH = 5.5]
C-6	131.88 [^{1}JCH = 192.3]	132.16 [^{1}JCH = 194.6 – ^{2}JCH = 2.1]
C-3	150.43	149.36 [^{3}JCH = 7.2]
C-4	155.01	154.46 [^{3}JCH = 7.9 – ^{2}JCH = 2.1]

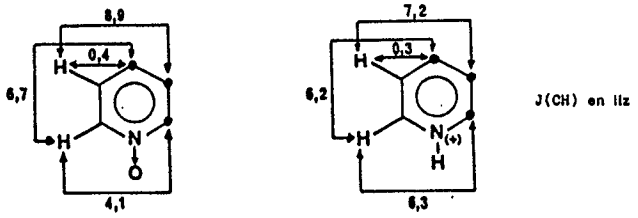
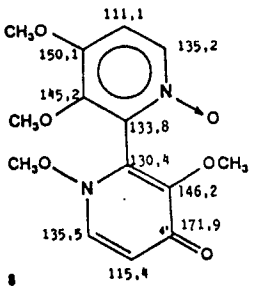


Figure 3

Long-range J[CH] coupling constants in pyridine N-oxide (12,16) and pyridinium cation (16, 17)



EXPERIMENTAL

The orellanine studied in this work was extracted from a sample of *Cortinarius orellanus* following described methods (2, 3, 19). It was found to be identical to a synthetic sample.

1D and 2D NMR spectra were recorded on a Bruker AC-250 spectrometer at 250.13 MHz (^{1}H) and 62.90 MHz (^{13}C) using the standard Bruker microprograms (XHCORR for 2D heteronuclear correlation spectra).

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