Phytochemistry, Vol. 28, No. 1, pp. 308–309, 1989.
Printed in Great Britain.

0031 9422/89 \$3.00+0.00
Pergamon Press plc.

THE COMPLETE ASSIGNMENT OF THE ^{13}C AND ^{1}H NMR SPECTRA OF JADIFFINE

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(*Received* 29 *February* 1988)

Key Word Index—*Vinca difformis*; Apocynaceae; jadiffine; alkaloid; ^{1}H homonuclear correlation; ^{13}C–^{1}H chemical shift correlation.

Abstract—The structure of jadiffine, a new alkaloid of *Vinca difformis* was elucidated by means of two dimensional NMR experiments: COSY, COSY long range and heteronuclear ^{1}H–^{13}C chemical shift correlation.

INTRODUCTION

This paper reports the determination of the structure of jadiffine (**1**). Jadiffine is a new alkaloid isolated from *Vinca difformis*. The MS of **1** shows a molecular peak at *m/z* 324 corresponding to a formula $C_{22}H_{24}N_2O_2$. The UV spectrum shows an indolic chromophore. A two-dimensional NMR study was undertaken to provide unambiguous ^{1}H and ^{13}C assignments. Moreover the ^{1}H coupling constants were estimated from a one-dimensional spectrum after identification of the patterns by the COSY technique.

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C NMR spectra of jadiffine (**1**) and of *O*-acetyl jadiffine show the presence of ketonic and primary alcoholic functions, an ethyl side chain, a *N*-methyl group and free indolic NH. Two dimensional NMR studies: homonuclear COSY and COSY LR, heteronuclear ^{13}C–^{1}H chemical shift correlation had allowed the structural determination of this alkaloid of macroline type structure [1]. For jadiffine the chemical shifts of C_3, C_{14} and C_{20} are very similar to those of villalstonine [2], macralstonidine [2] and pandicine [3]. However due to substitution on C_6, C_{21} is slightly deshielded.

In the ^{1}H NMR spectrum of **1** the chemical shift of H_3 (4.05 ppm) appears very close to that of the corresponding proton of pandicine and talcarpine [3] respectively 4.01 and 3.98 ppm. The C_6 and C_{16} signals appear at 48.0 and 50.8 ppm respectively. Those low shift values indicate the presence of an alpha keto group on C_5. COSY and irradiation experiments allow the assignment of H_{16}, H_{17} and H_{20}, H_{19} and H_{18}. The low coupling constants between H_{20} and H_{21}, H_{15} and H_{16} indicate the stereochemistry 16*R*,20*S*. The coupling constant between H_{15} and H_{20} is lower than 1 Hz. A long distance COSY experiment displays a correlation between H_{21} (3.45 ppm) and H_{15} (1.65 ppm). The above experimental data are in complete agreement with the geometry observed on molecular models: dihedral angles between H_{15}–H_{16}, H_{20}–H_{21} and H_{15}–H_{20} are close to 90°, H_{21} and H_{15} are in a *W* disposition.

Table 1. ^{1}H and ^{13}C chemical shifts (ppm) ^{1}H–^{1}H coupling constants (Hz) for jadiffine, **1**.

	^{1}H		^{13}C
Position	δ	J	δ
2			135.9
3	4.05	$J_{3-14a}=6$ $J_{3-14b}\simeq 1$	54.5
N-Me	2		43.2
5			215
6	4.25	$J_{6-21}=9$	48.0
7			104.6
8			126.3
9	7.47	$J_{9-10}=7$	118.3
10	7.07	$J_{10-11}=7$ $J_{10-12}=2$	120.6
11	7.13	$J_{9-11}=2$	122.2
12	7.30		111.5
13			135.9
N-H	8.02		
14	$14_a=2.60$ $14_b=1.77$	$J_{14a-14b}=12$ $J_{14a-15}\simeq 1$ $J_{14b-15}\simeq 1$	32.3
15	1.65	$J_{15-16}\simeq 1$ $J_{15-20}\simeq 1$	34.5
16	2.50	$J_{16-17a}=4$ $J_{16-17b}=9$	50.8
17	17a = 3.33 17b = 3.65	$J_{17a-17b}=9$	64.3
18	0.95	$J_{18-19}=7$	12.2
19	19a = 1.60 19b = 1.90	$J_{19a-19b}=12$	23.5
20	1.43	$J_{20-21}\simeq 1$	43.6
21	3.50		59.2

*The values of the chemical shifts of C_9 to C_{12} are similar to values of literature [6].

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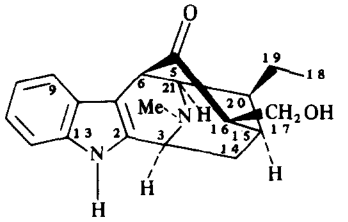

Jadiffine **1**

The heteronuclear ^1H–^{13}C chemical shift inverse correlated experiment [4] allows the complete assignment of carbons (Table 1).

EXPERIMENTAL

Jadiffine was isolated from *Vinca difformis* Pourr. (Apocynaceae) according to the operating process previously reported [5, 6]. It was purified by prep. CC (silica G, Merck) with CH_2Cl_2–MeOH (93/7). ^1H spectra of **1** was recorded on a Bruker AM 250 NMR spectrometer. Proton chemical shifts are given with reference to TMS as internal standard.

Homonuclear ^1H–^1H shift-correlated two-dimensional diagram was obtained using the COSY-45 pulse sequence (D_1, 90°, t_1, 45°, acquisition). Parameters are as follows: spectral widths $F_2 = 2500$ Hz, $F_1 = 1250$ Hz, data matrix for processing: 1024 $(f_2) \times 512$ (f_1), relaxation delay 0 sec, number of scans 32, increments of t_1: 256. The COSY LR sequence (D_1, 90°, t_1, D_2, 45°, D_2, acquisition) was used to favour cross-peaks relating weak protons signals and D_2 was set to 0.08 sec. The heteronuclear two-dimensional ^1H-^{13}C shift inverse correlated spectrum was recorded on a Bruker AM 400 spectrometer (program Birdrev Au). Parameters are as follows: spectral widths 8560 Hz (C13 axis) 3400 Hz (H, axis); data matrix for processing 4096 × 512; relaxation delay: 0 sec. number of scans: 16; increments of t_1: 64.

Acknowledgements—We thank Bruker Co. for recording the ^1H–^{13}C correlated spectrum.

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