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REVISION OF THE STRUCTURES OF RHAZICINE AND RHAZIMINE, TWO ALKALOIDS FROM *MELODINUS ACUTIFLORUS*

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Key Word Index—*Melodinus acutiflorus*; Apocynaceae; alkaloids; structure revision of rhazicine and rhazimine; relative configuration; crystal structure; spectral data.

Abstract—From the leaves of *Melodinus acutiflorus* the two alkaloids rhazicine and rhazimine were isolated and identified with known substances. However, based on extensive spectral and X-ray analysis their structures published earlier have to be revised.

INTRODUCTION

In the past, plants of the genus *Melodinus* (Apocynaceae, Carissae) have been investigated extensively for alkaloids. Some of these alkaloids are indole alkaloids, others are built up biogenetically from the same building blocks (tryptamine and secologanin) but contain a quinoline instead of an indole chromophore. To date, only nine members of this alkaloid type have been isolated [1]. These are meloscine [2], 16-*epi* meloscine [2], 16-*epi* meloscine-*N*-oxide [3], scandine [2], meloscandonine [4], 19-*epi* meloscandonine [5], scandomeline [5], 19-*epi* scandomeline [5] and lanceomigine [6]. Comparably derived quinoline alkaloids (e.g. quinine) are known from plants of the family of Rubiaceae. *M. acutiflorus* itself was not then screened for alkaloids.

RESULTS AND DISCUSSION

From the chloroform extract of the leaves of *M. acutiflorus* we have isolated the two alkaloids rhazicine (**1**) and its anhydro product rhazimine (**2**). During chromatography rhazicine converted to rhazimine by loss of one mol. of water. By treatment of **1** with acetic anhydride–pyridine a mixture of **1** and **2** was formed. Structure determination was performed on the main product, rhazicine (**1**), crystallized from MeOH and it gave the result shown in the Fig. 1 and Scheme 1.

Because of the structural changes between **1** [HO–C(2)–O–C(17)–N–] and **2** [C(2)=O C(17)=N–] which are in the neighbourhood of the chromophores, the UV spectra of **1** and **2** show significant differences (see Experimental). In the IR spectrum of **1** $\nu_{CO}^{CHCl_3}$ appears at 1742 and in that of **2** at 1740 and 1732 cm^{-1}.

The mass spectrum (EI mode) of **1** is characterized by the base peak at *m/z* 122 and a significant peak for the loss of water (*m/z* 350). The corresponding ion is further decomposed to *m/z* 322. Indicated by m* signals (linked scan technique) the latter is transformed to *m/z* 263 and 122. This principal fragmentation pattern is given in Scheme 2.

Using 2D-NMR methods we have been able to assign completely the ^1H and the ^{13}C spectra of **1**. The assignment of the signals in **2** was done on the basis of that of **1** and of additional decoupling experiments (see Tables 1, 2 and 3). Comparison of the ^{13}C spectra of **1** and **2** reveals close similarity except for C(2) and C(17) which differ

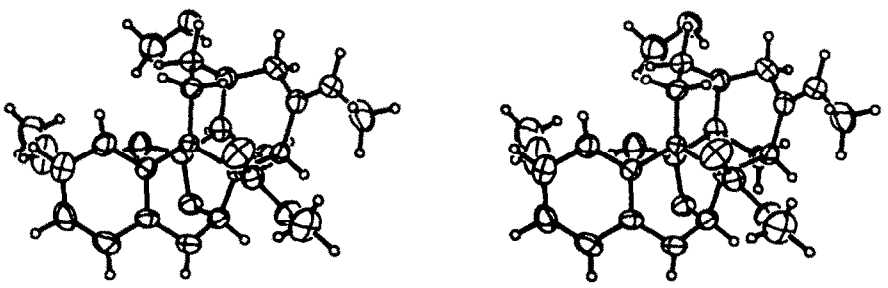
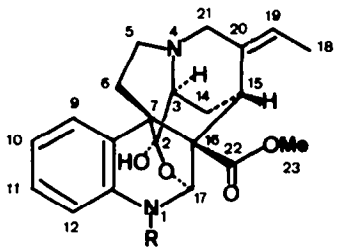
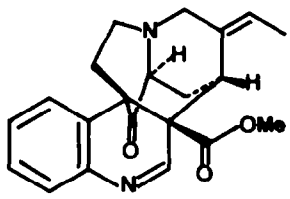


Fig. 1.

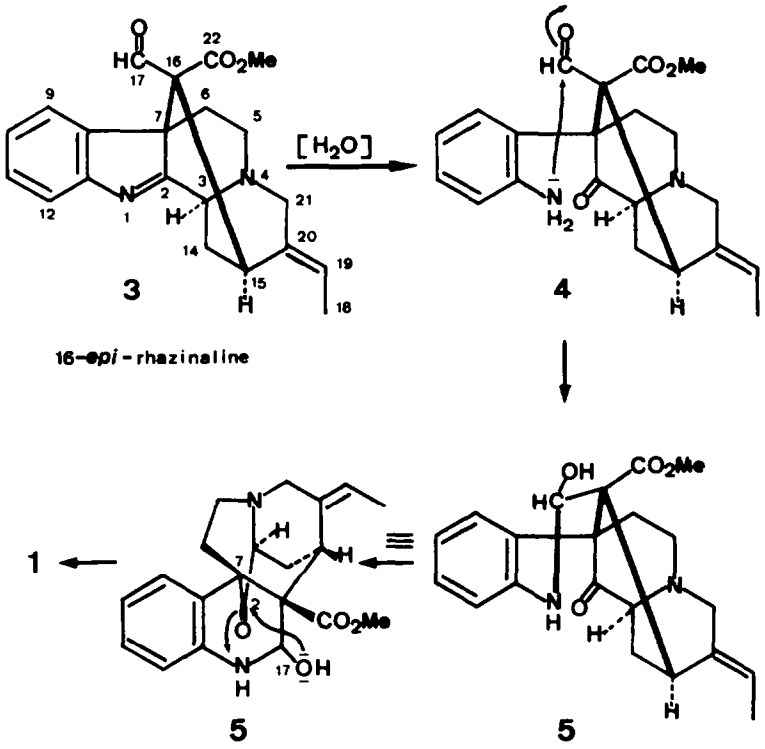


1 R=H, Rhazicine

8 R=Me, Lanceomigine



2 Rhazimine

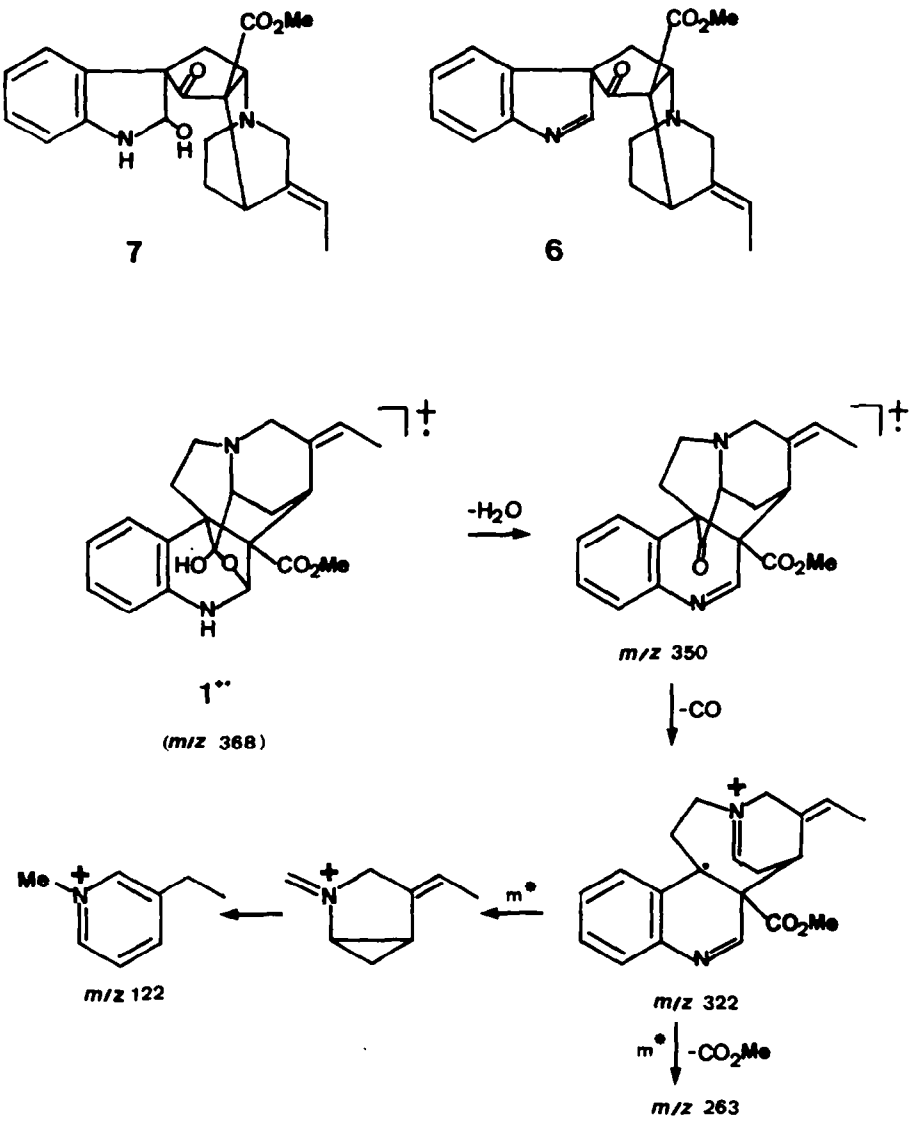


Scheme 1. Structure and possible biogenetic pathway of rhazicine (1).

from each other by more than 70 ppm. These findings are in full agreement with the structural differences between 1 and 2. On the basis of the extracted coupling constants for both compounds, the geometrical arrangement of 1 and 2 have to be similar and rhazimine must therefore possess structure 2.

The structure of rhazicine represents an interesting variation of *Melodinus* alkaloids. It can be assumed that the biogenetic pathway of rhazicine (1) begins with the known 16-*epi* rhazinaline (3) [7] which, under hydrolytic enzymatic conditions, is transformed to 4. This compound can undergo a ring closure reaction via the aminal 5 to rhazicine (1) by acetalization of the ketone group (Scheme 1). X-ray structure determination gave the relative configuration of 1. If the postulated biogenetic pathway is correct and 16-*epi* rhazinaline is the precursor of 1, its structure represents the absolute configuration. It was demonstrated that all indole alkaloids of type 3 have the same absolute configuration at C(15) [8].

The names rhazicine and rhazimine for the two alkaloids isolated from *M. acutiflorus* are the same as used for alkaloids isolated from *Rhazya stricta* Decaisne. The alkaloid sample of rhazimine (2) isolated from *R. stricta* [9] kindly provided by Atta-ur-Rahman and that of *M. acutiflorus* show the same behavior on TLC (silica gel: $CHCl_3$–MeOH–HOAc, 8:9:1, $R_f = 0.50$; Et_2O–MeOH–aq. NH_4OH, 80:19:1, $R_f = 0.47$; EtOAc, $R_f = 0.29$), the same colour reaction with $Ce(SO_4)_2$ reagent, the same EI mass spectra and the same 400 MHz ^1H NMR spectra. Therefore, both compounds are identical. The structure of rhazimine given in [9] as 6 is incorrect and has to be changed to 2. Because of the



Scheme 2. Mass spectral fragmentation of rhazicine (1).

correlation of 2 and 1 (see above), the structure of rhazicine given in [10] as 7 must be changed to 1. We have to assume that 2-methoxy-1,2-dihydrorhazimine [11] has to be corrected, too.

An alkaloid called lanceomigine was isolated from *Alstonia lanceolata* V. Heurck et Müll. Arg. [12]. Its structure was given as *N*(1)-methyl-rhazicine (8). The structure determination was based on extensive spectral analysis and on an X-ray structure determination of a derivative [13]. Methylation (H_2CO–HOAc–$NaCNBH_3$) of rhazicine (1) gave a product which shows great similarity with the published spectral data of lanceomigine [6].

EXPERIMENTAL

^{1}H NMR spectra were recorded at 400 and ^{13}C NMR at 50 MHz, 2D experiments at 400 MHz, using the $CHCl_3$ signal as ref.; UV spectra were recorded in 95% EtOH, IR spectra in $CHCl_3$ and EIMS were measured at 70 eV.

Plant material. M. acutiflorus S. V. Müll. was collected near Brisbane/Australia.

Extraction and chromatography. Dried leaves (500 g) were extd × 4 with 3% MeOH–HOAc at room temp. The MeOH extract was evapd (< 40°), dil. with 0.25 N aq HCl and extd several times with Et_2O to remove the non-basic compounds. The aq phase was basified with Na_2CO_3 to pH 7, extd with Et_2O (AM1) and $CHCl_3$ (AM2), and continued to be basified to pH 9, extd with $CHCl_3$ (AM3). The organic soln was evapd (< 40°). After chromatography on silica gel using $CHCl_3$–MeOH–NH_4OH (variating ratios) we obtained from AM2 and AM3 600 mg of compound (1) and small amounts of (2).

Rhazicine (1). Crystallized from MeOH in colourless prisms, mp 122.6–124.4°; $[\alpha]_D^{22}$ + 69.8° ($CHCl_3$, c = 0.76); pink colour with $Ce(SO_4)_2$. UV λ_{max} nm: 208 (log ε 4.16), 249 (3.70), 294 (3.22), λ_{min} nm: 228 (3.34), 272 (2.85); IR ν_{max} cm^{-1}: 3630, 3525,

Table 1. ^{13}C NMR spectral data ($CDCl_3$) of rhazicine (1) and rhazimine (2)

Carbon	1	2
2	111.12 *s*	214.51 *s*
3	57.40 *d*	61.17 *d*
5	48.31 *t*	51.65 *t*
6	23.10 *t*	30.39 *t*
7	49.72 *s*	53.14 *s*
8	126.19 *s*	137.43 *s*
9	127.29 *d*	128.36 *d*[a]
10	119.41 *d*	128.01 *d*[a]
11	128.44 *d*	128.84 *d*[a]
12	115.81 *d*	120.76 *d*
13	142.52 *s*	142.03 *s*
14	27.65 *t*	32.16 *t*
15	36.89 *d*	37.35 *d*
16	57.40 *s*	58.09 *s*
17	84.46 *d*	160.60 *d*
18	12.62 *q*	13.03 *q*
19	118.18 *d*	125.05 *d*[a]
20	141.08 *s*	137.43 *s*
21	54.14 *t*	53.04 *t*
22	170.91 *s*	168.42 *s*
23	51.69 *q*	52.16 *q*

[a]Interchangeable.

3415 (NH and OH bands), 1742 (ester band), 1725, 1609 (tetrahydroquinoline), 1476. ^{1}H NMR and ^{13}C NMR see Tables. CIMS (2-methylpropane): 369 ($[M+1]^+$), 351 ($[M+1-18]^+$). EIMS: *m/z* (rel. int.): 368 (8, $[M]^{+\cdot}$), 350 (15), 322 (16), 277 (6), 263 (12), 214 (11), 180 (10), 169 (10), 154 (14), 122 (100), 121 (21), 108 (11).

X-ray diffraction analysis of rhazicine·2 MeOH at room temp. Formula of the asymmetric unit $C_{21}H_{24}N_2O_4$·2 MeOH, space group $P2_12_12_1$; $a = 7.649(1)$, $b = 15.671(1)$, $c = 18.152(1)$ Å [from a least squares fit to the 2θ-values of 92 automatically centred reflections in the range $26 < |2\theta| < 33°$, $\lambda(MoK_\alpha) = 0.71069$ Å]. 2836 symmetry independent reflections within $\lambda^{-1}\cdot\sin\theta < 0.65$ Å were measured on a Nicolet R3 diffractometer using the ω-scan technique and were corrected for Lorentz and polarisation effects but not for absorption. The structure was solved by direct methods [14]. All H atoms were found by a difference density calculation and were freely refined with isotropic temperature factors, an exception being the H atoms in the methyl group of one MeOH molecule for which the riding model with $U = 0.2$ Å was used. All other atoms were freely refined with anisotropic temperature factors. The 2390 structure factors with $F > \sigma_F$ were used in a blocked cascade least squares process (*ca* 100 variables per block) [14] to refine the 396 variables to $R = 0.101$, $wR = 0.053$ for all 2836 reflections, $w = (\sigma_F^2 + 0.0004F^2)^{-1}$. The e.s.d.s. of the bond lengths range from 0.004 to 0.007 Å. The absolute structure was not determined. For atom coordinates and temperature factors see Table 4.

Table 2. ^{1}H NMR spectral data of rhazicine (1) (400 MHz)

Hydrogen	δ(ppm)	Multiplicity	J(Hz)
1*	4.83	*d*	$J_{(1,17)} = 4.4$
3	3.56	*dd*†	$J_{(3,14\alpha)} = 3.4$, $J_{(3,14\beta)} = 2.4$
5α	3.56	*dt*	$J_{(5\alpha,5\beta)} = J_{(5\alpha,6\beta)} = 13.8$, $J_{(5\alpha,6\alpha)} = 3.8$
5β	2.76	*dd*	$J_{(5\beta,5\alpha)} = 13.8$, $J_{(5\beta,6\beta)} = 5.3$
6α	2.23	*dd*	$J_{(6\alpha,6\beta)} = 16.2$, $J_{(6\alpha,5\alpha)} = 3.8$
6β	2.92	*ddd*	$J_{(6\beta,6\alpha)} = 16.2$, $J_{(6\beta,5\alpha)} = 13.8$, $J_{(6\beta,5\beta)} = 5.3$
9	7.30	*dd*	$J_{(9,10)} = 7.7$, $J_{(9,11)} = 1.1$
10	6.87	*dt*	$J_{(10,9)} = J_{(10,11)} = 7.7$, $J_{(10,12)} = 1.1$
11	7.11	*dt*	$J_{(11,10)} = J_{(11,12)} = 7.7$, $J_{(11,9)} = 1.1$
12	6.57	*dd*	$J_{(12,11)} = 7.7$, $J_{(12,10)} = 1.1$
14α	2.04	*ddd*	$J_{(14\alpha,14\beta)} = 13.9$, $J_{(14\alpha,15)} = 1.9$, $J_{(14\alpha,3)} = 3.4$
14β	2.32	*ddd*	$J_{(14\beta,14\alpha)} = 13.9$, $J_{(14\beta,3)} = 2.4$, $J_{(14\beta,15)} = 4.1$
15	3.62	*ddd*†	$J_{(15,19)} = 1$, $J_{(15,14\alpha)} = 1.9$, $J_{(15,14\beta)} = 4.1$
17	4.91	*d*	$J_{(17,1)} = 4.4$
18(3H)	1.49	*dd*	$J_{(18,19)} = 7.1$, $J_{(18,21\alpha)} = 2.5$
19	5.38	*ddq*	$J_{(19,18)} = 7.1$, $J_{(19,21\alpha)} = 2.5$, $J_{(19,15)} = 1$
21α	3.97	*dquint*	$J_{(21\alpha,21\beta)} = 16.5$, $J_{(21\alpha,19)} = J_{(21\alpha,18)} = 2.5$
21β	2.93	*d*	$J_{(21\beta,21\alpha)} = 16.5$
23(3H)	3.57	*s*	
2(OH)*	3.03	*s(br)*	

The assignments of the α and β protons at C(14) and C(21) in 1 were made on the basis of homonuclear NOE experiments.

*With D_2O interchangeable.

†The multiplicity and the coupling constants of these signals were determined by indirect methods.

Table 3. ^{1}H NMR spectral data of rhazimine (2) (400 MHz)

Hydrogen	δ(ppm)	Multiplicity	J(Hz)
3	3.91	*d*	$J_{(3,14\beta)} = 5.3$
5α	3.78	*ddd*	$J_{(5\alpha,5\beta)} = 15.6$, $J_{(5\alpha,6\beta)} = 14.4$, $J_{(5\alpha,6\alpha)} = 5.8$
5β	2.80	*dd*	$J_{(5\beta,5\alpha)} = 15.6$, $J_{(5\beta,6\beta)} = 3.5$
6α	2.99	*dd*	$J_{(6\alpha,6\beta)} = 14.4$, $J_{(6\alpha,5\alpha)} = 5.8$
6β	3.06	*dt*	$J_{(6\beta,6\alpha)} = J_{(6\beta,5\alpha)} = 14.4$, $J_{(6\beta,5\beta)} = 3.5$
9–12	7.4–7.3	*m*	
14α	2.11	*dd*	$J_{(14\alpha,14\beta)} = 14.8$, $J_{(14\alpha,15)} = 2.5$
14β	2.48	*ddd*	$J_{(14\beta,14\alpha)} = 14.8$, $J_{(14\beta,3)} = 5.3$, $J_{(14\beta,1)} = 2.7$
15	3.76	*dd**	$J_{(15,14\alpha)} = 2.5$, $J_{(15,14\beta)} = 2.7$, $J_{(15,19)} = 2$
17	7.70	*s*	
18(3H)	1.59	*dd*	$J_{(18,19)} = 7.1$, $J_{(18,21\beta)} = 2.5$
19	5.58	*ddq*	$J_{(19,18)} = 7.1$, $J_{(19,21\beta)} = 2.5$, $J_{(19,15)} = 2$
21α	4.12	*d quint*	$J_{(21\alpha,21\beta)} = 17.3$, $J_{(21\alpha,18)} = J_{(21\alpha,19)} = 2.5$
21β	3.25	*d*	$J_{(21\beta,21\alpha)} = 17.3$
23(3H)	3.52	*s*	

*The multiplicity and the coupling constants of this signal were determined by indirect methods.

Table 4. Atom coordinates (× 10^4) and temperature factors (Å^2 × 10^3) for rhazicine (1) dimethanol solvate

Atom	x/a	y/b	z/c	U_{eq}*
N(1)	3112(4)	6253(2)	3789(2)	42(1)
C(2)	5444(5)	4988(2)	2763(2)	34(1)
O(2)	6847(3)	5330(2)	2391(1)	47(1)
C(3)	5020(5)	4114(2)	2407(2)	38(1)
N(4)	6148(4)	3408(2)	2698(2)	40(1)
C(5)	7540(4)	3707(2)	3196(2)	40(1)
C(6)	6864(4)	4186(2)	3858(2)	35(1)
C(7)	5604(4)	4897(2)	3615(2)	28(1)
C(8)	6122(4)	5784(2)	3914(2)	31(1)
C(9)	7824(5)	6000(2)	4080(2)	39(1)
C(10)	8307(5)	6812(3)	4302(2)	48(1)
C(11)	7062(6)	7425(2)	4365(2)	51(1)
C(12)	5327(5)	7253(2)	4192(2)	47(1)
C(13)	4869(5)	6434(2)	3961(2)	35(1)
C(14)	3096(5)	3939(2)	2551(2)	39(1)
C(15)	2893(4)	3876(2)	3396(2)	33(1)
C(16)	3612(4)	4721(2)	3760(2)	28(1)
C(17)	2905(5)	5501(2)	3349(2)	37(1)
O(17)	3948(3)	5550(1)	2683(1)	38(1)
C(18)	1831(8)	2698(4)	4711(3)	63(2)
C(19)	3251(5)	2550(2)	4157(2)	45(1)
C(20)	3735(5)	3045(2)	3609(2)	37(1)
C(21)	5093(5)	2725(2)	3057(2)	46(1)
C(22)	3172(5)	4749(2)	4575(2)	38(1)
O(22)	4184(4)	4690(2)	5070(1)	59(1)
C(23)	839(7)	4907(4)	5421(3)	69(2)
O(23)	1431(3)	4817(2)	4672(1)	47(1)
O(M1)	8579(4)	2883(2)	1685(2)	55(1)
C(M1)	9019(8)	3627(4)	1249(3)	64(2)
O(M2)	9202(4)	6664(2)	2517(2)	86(1)
C(M2)	10144(6)	6438(3)	1868(2)	75(2)

*Equivalent isotropic *U* defined as one third of the trace of the orthogonalized *U* tensor.

Rhazimine (2). Crystallized from MeOH, mp 170.5° (dec.); $[\alpha]_D^{22} = +252.0°$ ($CHCl_3$, *c* 0.23); pink colour with $Ce(SO_4)_2$; UV λ_{max} nm: 202 nm (log ε 4.30), 218 (4.22), 260 (3.63), 288 (sh), λ_{min} nm: 214 (4.21), 251 (3.62), IR λ_{max} cm^{-1}: 1740 (ester), 1732 (ketone), 1628; ^{1}H NMR and ^{13}C NMR see Tables. EIMS *m/z* (rel. int.): 350 (4, $[M]^+$), 322 (5), 307 (1), 291 (1), 263 (2), 214 (4), 122 (100), 108 (9), 93 (6), 77 (5).

Rhazimine (2) *from rhazicine* (1). Treatment of a sample of (1) with Ac_2O–pyridine gave a mixture of rhazimine (2) as main product and starting material; identification by TLC. During chromatography (silica gel; $CHCl_3$–MeOH–NH_4OH) of (1) rhazimine was formed in small quantities.

Conversion of rhazicine (1) *to lanceomigine* (8). 1 (10 mg) was dissolved in a soln of HCHO (5 ml, 40% in H_2O) and HOAc (5 drops). Afterwards at 20°, $NaCNBH_3$ was added slowly under stirring. After 15 min the reaction mixt was poured into ice-H_2O, basified with satd aq $NaHCO_3$, extd with $CHCl_3$, dried and evapd. Chromatography of the residue gave 8. Pink colour with $Ce(SO_4)_2$. ^{1}H NMR: 5.48 (1H, *q*, H-19), 4.82 (1H, *s*, H-17), 3.57 (3H, *s*, O–CH_3), 2.98 (3H, *s*, N–CH_3), 1.51 [3H, *dd*, CH_3 (18)]. EIMS *m/z* (rel. int.): 382 (40, $[M]^+$), 367, 338, 337, 323, 279, 222, 207, 194, 167, 158, 157, 122 (100), 121.

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