THE PYRROLIZIDINE ALKALOIDS FROM SENECIO RACEMOSUS

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Abstract-- Two new pyrrolizidine alkaloids, racemocine and racemonine, have been isolated from Senecio racemosus. Their structures (1 and 2) have been assigned on the basis of spectral studies including 2D-nmr.

The Senecio species (Compositae) are considered to be a rich source of pyrrolizidine alkaloids, some of them possess antitumor, carcinogenic, hepatotoxic, and mutagenic activities.[1-4] The wide range of biological activities exhibited by these alkaloids prompted us to carry out systematic chemical studies on Senecio racemosus which is widely distributed in the temperate regions and in the hills of tropics. The literature survey shows that only one pyrrolizidine alkaloid, senecionphylline,[5] has so far been reported from this plant. As a result of our investigation on the fresh and undried plant material of Senecio racemosus we have isolated two new pyrrolizidine alkaloids namely recemocine and racemonine to which the structures (1) and (2) have been assigned on the basis of extensive nmr studies including homonuclear 2D ^{1}H-nmr (COSY-45°, J-resolved), heteronuclear ^{1}H-^{13}C correlated spectroscopy (Heterocosy), ^{13}C-nmr and DEPT experiments.

RESULTS AND DISCUSSION

Racemocine (1) was isolated as a gummy solid. $[\alpha]_D^{20}$ = -16° (c = 0.25, $CHCl_3$). Its uv spectrum showed absorption at 230 nm. The ir spectrum indicated the presence of OH group at 3300 cm^{-1}, conjugated ester function at 1690 cm^{-1}, and conjugated double bond at 1630 cm^{-1}. The high resolution mass spectrum (hrms) gave the molecular ion peak at m/z 239.1494 corresponding to the molecular formula $C_{13}H_{21}NO_3$ indicating four degrees of unsaturation in the molecule. The molecular ion peak was also authenticated by field desorption mass spectrum (fdms). The hrms further showed prominent peaks at m/z 224.1434 ($C_{12}H_{18}NO_3$) and m/z 221.1399 ($C_{13}H_{19}NO_2$) resulting from the loss of methyl group and water respectively. While the ions at m/z 156.1029 ($C_8H_{14}NO_2$) and m/z 139.0989 ($C_8H_{13}NO$) represented the loss of angelic acid and angeloyl

moieties. The remaining peaks at m/z 113.0915 ($C_6H_{11}NO$), m/z 99.0383 (C_5H_9NO) and m/z 82.0220 (C_5H_8N) (base peak) were characteristic of ring saturated pyrrolizidine alkaloids.[6]

The ^{13}C-nmr spectrum ($CDCl_3$, 75 MHz) showed thirteen carbon atoms. The multiplicity assignments were determined by using DEPT experiments with the polarization pulse θ = 45°, 90°, and 135°. These experiments revealed two methyl, five methylene, four methine and two quaternary carbon atoms.

The ^{1}H-nmr spectrum of racemocine showed the presence of angelic ester moiety[7] (1H, quartet quartet at δ 6.17, J = 7.1, 1.40 Hz, 3H doublet quartet at δ 1.97, J = 7.1, 1.49 Hz, 3H doublet quartet at δ 1.90, J = 1.49, 1.40 Hz). Apart from that three methine protons were observed at δ 4.70, 4.31, and 2.98, respectively. Of these the most downfield signal was assigned to the proton geminal to the hydroxyl group, while the signal at δ 4.31 could be attributed to H-8. The multiplet at δ 2.98 was characteristic of H-1 in platynecine type of bases carrying variety of ester functions.[7] Among the methylene groups, the most downfield signals at δ 4.52 and 4.63 (J_{gem} = 10.5 and J_{vic} = 7.3 Hz) were again in complete agreement for H_2-9 of platynecine type bases. In ^{1}H-^{1}H correlated spectroscopy cross peaks were observed between H-1, H-8 and H-7, H-8 respectively. On the other hand, H-7 further showed connectivities to protons at δ 2.15 and δ 2.40, both of which were found to be part of a methylene group through heterocosy. Similar type of cross peaks were observed between H-1 and methylene protons at δ 2.24 and δ 2.47, respectively. The remaining one proton signals at δ 3.82 and 4.18 were assigned to methylene protons adjacent to nitrogen at C-3 while both the protons at C-5 occurred together at δ 3.80. The assignments were further confirmed by cosy spectrum. From the foregoing the structure of racemocine appeared to be similar with that of 9-angelylplatynecine.[7] In so far, however, as the two bases show differences in chemical shifts of some signals in the ^{1}H-and ^{13}C-nmr spectra, the former is evidently a stereoisomer of the latter. Careful comparison of ^{1}H-nmr of the bases with respect to protons at assymetric centers, the chemical shifts of H-1 of the two bases showed very close agreement revealing similarity in stereochemistry at this center. On the other hand, the signals of H-7 and H-8 of racemocine were shifted downfield by 0.78 and 0.36 ppm, respectively, as compared to the 9-angelylplatynecine. Similar downfield shifts were observed for C-7 and C-8 in ^{13}C-nmr spectra of the two compounds.[8] The downfield shift of H-8 and H-7 could only be justified by assuming β orientations as against α orientations in angelylplatynecine. Such trends in nmr spectra have already been observed in literature for bases differing in stereochemistry[9] at these centers. Further insight to stereochemistry was provided by noesy spectrum. The H-7 showed cross peaks with H-8 as well as H-6. On the other hand, H-8 showed only one cross peak with H-7. Conclusive evidence to the stereostructure 1 of racemocine was provided by NOE difference measurements. Iraddidation at δ 4.31 (H-8)

caused 24% enhancement in the signal of H-7 at δ 4.70 while irradiation at δ 4.70 caused 22% enhancement in the signal of H-8 and 16% enhancement in the signal of H-6, at δ 2.40.

Racemonine (2) formed a gummy solid, $[\alpha]_D^{20}$ = -24.3° (c = 0.205, $CHCl_3$). Its uv spectrum showed absorption at 230 nm. The ir spectrum indicated the presence of OH group at 3300 cm^{-1}, non-conjugated ester at 1720 cm^{-1}, conjugated ester function at 1690 cm^{-1} and conjugated double bond at 1630 cm^{-1}. The high resolution mass spectrum (hrms) gave the molecular ion peak at m/z 311.1687 corresponding to the molecular formula $C_{16}H_{25}NO_5$ indicating five degrees of unsaturation in the molecule. The molecular ion peak was also authenticated by field desorption mass spectrum (fdms). The hrms further showed the prominent peaks at m/z 297.1796 ($C_{16}H_{23}NO_4$), and m/z 239.1494 ($C_{13}H_{21}NO_3$) resulting from the loss of water and propionate moiety, respectively. The remainings peaks at m/z 113.0915 ($C_6H_{11}NO$), 99.0383 (C_5H_9NO), and 82.0220 (C_5H_8N) (base peak) were characteristic of saturated pyrrolizidine alkaloids.[6]

The ^{13}C-nmr spectrum ($CDCl_3$,75 MHz) showed sixteen carbon atoms. The multiplicity assignments were determined by using DEPT experiments with the polarization pulse θ = 45°, 90°, and 135°. These experiments revealed three methyl, six methylene, three methine and four quaternary carbon atoms.

The ^{1}H-nmr spectrum of racemonine showed the presence of 2-propionyloxysenecic acid ester moiety (6H singlet at δ 1.90, 3H triplet at δ 1.18, J = 7.1 Hz and 2H quartet at δ 3.6, J = 7.1 Hz). Apart from that further three methine protons were observed at δ 4.71, 4.32 and 2.99. These could be assigned to the proton geminal to hydroxyl group, H-8, and H-1, respectively, as in the case of racemocine. The ^{1}H-^{1}H correlated spectroscopy showed all connectivities that were observed earlier for racemocine and helped us to assign all signals of the nucleus. The chemical shifts of the nucleus protons and carbons in ^{1}H- and ^{13}C-nmr spectra were in very close agreement for those of racemocine revealing similarity in structure and stereochemistry, the only difference being that of ester moiety. This could further be confirmed by similar connectivities in noesy spectra of the two compounds and similar magnitude of enhancement of various signals during NOE difference measurements. The stereostructure of racemonine could, therefore, be assigned as 2.

Although a variety of ester moieties have been reported on C-9 but the ester moiety of racemonine has not so far been reported from any of the necine bases and its isolation is of biogenetic importance.

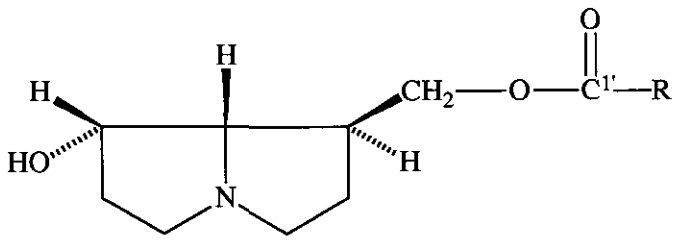
Table-1: Heterocosy[a] of 1 and 2

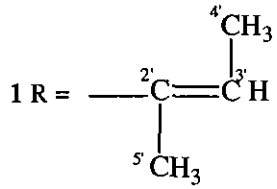
	Compound(1)[b]		Compound(2)[b]	
Carbon No.	^{13}C-Nmr	^{1}H-Nmr	^{13}C-Nmr	^{1}H-Nmr
1	41.3	2.98	41.0	2.99
2	29.9	2.24,2.47	29.8	2.23,2.46
3.	60.2	3.82,4.18	61.5	3.80,4.17
5	61.5	3.80	62.0	3.85
6	36.1	2.15,2.40	36.1	2.14,2.41
7	73.0	4.70	72.9	4.71
8	71.2	4.31	71.0	4.32
9	63.3	4.63,4.52	63.3	4.62,4.55
1'	169.0	--	169.1	--
2'	128.5	--	144.2	--
3'	139.6	6.17	138.7	--
4'	16.0	1.97	24.1	1.90
5'	20.8	1.90	20.7	1.90
1"	--	--	179.3	--
2"	--	--	58.3	3.64
3"	--	--	18.3	1.18

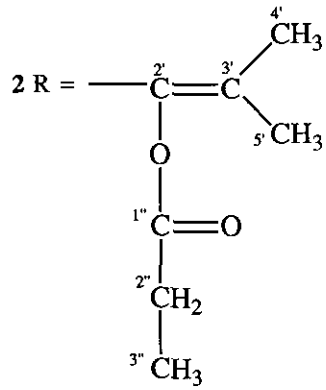
a) Chemical shifts are in δ ppm with reference to TMS as internal standard.

b) Solvent $CDCl_3$.

The alkaline hydrolysis of either racemocine (**1**) or racemonine (**2**) with alcoholic KOH [1 g KOH in 10 ml mixture of water-ethanol (1:2)] afforded a new necine base racemonecine (**3**) which was crystallized from acetone to colorless needles, M^+ peak at m/z 157.1245, mp 138-40°C, $[\alpha]_D^{20}$ = -11.37° (c=0.21, $CHCl_3$). A careful comparison of physical constants and ^{1}H-nmr data of **3** with that of platynecine[7] suggested that recemonecine is an isomer of platynecine with different stereochemistry at C-7 and C-8 centers, further confirming the structures of recemocine and racemonine as **1** and **2**, respectively.







EXPERIMENTAL

The spectra were recorded on a Shimadzu uv-240 spectrophotometer and ir spectra were recorded on JASCO A-302 spectrophotometer. The hrms were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. The nmr spectra were recorded on a Bruker AM-300 spectrometer with TMS as internal reference. Tlc experiments were performed on silica gel (GF-254, 0.2 mm) cards (Riedel-De Haen) and Flash chromatograph: 230-400 mesh size. Two dimensional COSY-45° experiment was acquired at 300 MHz with sweep width of 4000 Hz (2K data points in ω_2) and 2000 Hz (256 t_1 values zero-filled to 1k) in ω_1. The heteronuclear two dimensional ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with sweep width of 12820 Hz (2K data points in ω_2) and 1024 Hz (256 t_1 values zero-filled to 1k) in ω_1. In both the 2D experiments a 2 sec. relaxation delay was used and 16 transients were performed for each t_1 values.

Isolation of Racemocine (1) and Racemonine (2)

The crude methanolic extract (1.5 kg) from the fresh and undried plant material of Turkish origin was partitioned between water (2 *l*) and hexane (10 *l*). The aqueous layer which gave positive Dragendorff's test was basified with 20% ammonia solution and the liberated alkaloids were extracted out with chloroform. The crude alkaloidal residue (2 g) recovered from the chloroform layer was subjected to initial fractionation by column chromatography using silica gel as adsorbent. The elution was carried out with increasing order of polarity gradients using hexane, chloroform and methanol. The major alkaloidal fraction was eluted with chloroform-methanol (8:2).

It was subjected to vacuum liquid chromatography using mixture of chloroform and methanol in increasing order of polarity as eluent. The eluate obtained from chloroform-methanol (7.5:2.5) was mainly a mixture of two alkaloids which was separated by flash column chromatography over silica gel using same solvent mixture. The fraction obtained with chloroform-methanol (8.5:1.5) was almost pure with traces of impurities which could be removed by preparative tlc on silica gel cards with chloroform-methanol (8:2) as solvent system to yield pure racemocine (1) (R_f=0.5) as a gummy solid (25 mg).

The eluate obtained with chloroform-methanol (8:2) was similarly subjected to preparative tlc using chloroform-methanol (7.5:2.5) as solvent system. Racemonine (2) formed a gummy solid (R_f = 0.45) (20.5 mg).

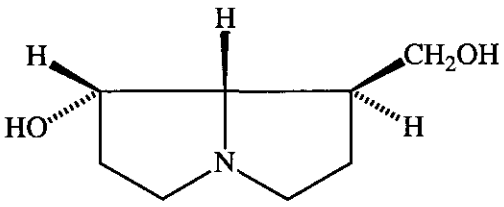
Racemocine (1): $[\alpha]_D^{20}$ -16° (c, 0.25, $CHCl_3$). Uv (CH_3OH): λ_{max} 230 nm. Ir ($CHCl_3$): ν_{max} 3300 cm^{-1} (hydroxy group), 1690 cm^{-1} (conjugated ester function), 1630 cm^{-1} (conjugated double bond). Hrms: M^+ 239.1494 ($C_{13}H_{21}NO_3$). Ms: m/z (rel. intens.) 239 (4), 224 (2), 221 (10), 156 (20), 139 (11), 113 (25), 99 (30), 96 (30), 95 (58), 83 (24), 82 (100), 80 (6) and 55 (8). ^{1}H-Nmr ($CDCl_3$, 300 MHz, δ ppm): 6.17 (1H, qq, J = 7.1, 1.40 Hz, C-3'H), 1.97 (3H, dq, J = 7.1, 1.49 Hz, C-4'H), 1.90 (3H, dq, J = 1.49, 1.40 Hz, C-5'H), 2.98 (1H, m, C-1H), 2.24 and 2.47 (2H, m, C-2H), 3.82 and 4.18 (2H, m, C-3H), 3.80 (2H, m, C-5H), 2.15 and 2.40 (2H, m, C-6H), 4.70 (1H, m, C-7H), 4.31 (1H, dd, J = 8.12, 2.5 Hz, C-8H), 4.63 (1H, dd, J_{gem} = 10.5 Hz, J_{vic} = 7.3 Hz, C-9H), 4.52 (1H, dd, J_{gem} = 10.5 Hz, J_{vic} = 7.3 Hz, C-9H). ^{13}C-Nmr ($CDCl_3$, 75 MHz) (δ ppm): 41.3 (C-1), 29.9 (C-2), 60.2 (C-3), 61.5 (C-5), 36.1 (C-6), 73.0 (C-7), 71.2 (C-8), 63.3 (C-9), 169.0 (C-1'), 128.5 (C-2'), 139.6 (C-3'), 16.0 (C-4'), 20.8 (C-5').

The assignments were made by comparison with published ^{13}C-nmr spectra of related bases[7,10] and confirmed in each case by ^{1}H-^{13}C correlated spectroscopy (Heterocosy).

Racemonine (2): $[\alpha]_D^{20}$ -24.3° (c, 0.205, $CHCl_3$). Uv (MeOH): λ_{max} 230 nm. Ir ($CHCl_3$): ν_{max} 3300 cm^{-1} (hydroxy group), 1720 cm^{-1} (non-conjugated ester), 1690 cm^{-1} (conjugated ester),

1630 cm^{-1} (conjugated double bond). Hrms: M^+ 311.1687 ($C_{16}H_{25}NO_5$). Ms: m/z (rel.intens.) 311 (0.4), 297 (5), 239 (1), 113 (30), 95 (92), 82 (100) and 55 (58). ^{1}H-Nmr ($CDCl_3$, 300 MHz, δ ppm): 1.90 (6H, s, C-4',5'H), 1.18 (3H, t, J = 7.1 Hz,C-3"H), 3.64 (2H, q, J = 7.1 Hz, C-2"H), 2.99 (1H, m, C-1H), 2.23 and 2.46 (2H, m, C-2H), 3.80 and 4.17 (2H, m, C-3H), 3.85 (2H, m, C-5H), 2.14 and 2.41 (2H, m, C-6H), 4.71 (1H, m, C-7H), 4.32 (1H, dd, J = 8.10, 2.4 Hz, C-8H), 4.62 (1H, dd, J_{gem} = 10.1 Hz, J_{vic} = 7.09 Hz, C-9H), 4.55 (1H, dd, J_{gem} = 10.1 Hz, J_{vic} = 7.09 Hz, C-9H). ^{13}C-Nmr ($CDCl_3$, 75 MHz) (δ ppm): 41.0 (C-1), 29.84 (C-2), 61.52 (C-3), 62.0 (C-5), 36.12 (C-6), 72.97 (C-7), 71.0 (C-8), 63.34 (C-9), 169.15 (C-1'), 144.22 (C-2'), 138.73 (C-3'), 24.19 (C-4'), 20.71 (C-5'), 179.35 (C-1"), 58.31 (C-2"), 18.36 (C-3"). The assignments were made by comparison with published ^{13}C-nmr spectra of related bases[7,10] and confirmed in each case by ^{1}H-^{13}C correlated spectroscopy (Heterocosy).

Racemocine (12 mg) and racemonine (15 mg) were separately refluxed for 15 minutes with alcoholic KOH [1 g KOH in 10 ml mixture of water-ethanol (1:2)]. The solvent was evaporated and the residue extracted with ether which on evaporation and crystallization from acetone gave colorless needles, mp 138-40°C, $[\alpha]_D^{20}$ = -11.37° (c=0.21, $CHCl_3$), M^+ 157.1245. ^{1}H-Nmr ($CDCl_3$, 300 MHz, δ ppm): 2.65 (1H, m, C-1H), 4.69 (1H, m, C-7H), 4.32 (1H, dd, J=8.11, 2.5 Hz, C-8H), 3.75 (2H, d, J=7.2 Hz, C-9H_2). ^{13}C-Nmr ($CDCl_3$, 75 MHz, δ ppm): 45.92 (C-1), 73.12 (C-7), 71.45 (C-8), 63.45 (C-9).



Racemonecine (3)

REFERENCES

1. F. L. Warren, "The Alkaloids", ed. R. H. F. Manske, Vol.12, Chapter 14, Academic Press, New York, 1970.
2. D. H. G. Crout, "The Alkaloids" (Specialist Periodical Report), Vol.6, Chapter 4, The Chemical Society London.
3. J. W. Cook, E. Duffy, and R. Schoental, Primary Liver Tumour Following Feeding with Alkaloids of Senecio jacobea, Brit. J. Cancer, 1950, 4, 405.
4. S. M. Kupchan, R. W. Doskotch, and P. W. Vanevenhoven, Tumour Inhibitors III. Monocrotaline, the Active Principle of Crotalaria spectabilis. J. Pharm. Sci., 1964, 53, 343.
5. M. P. Khamel, Pharmatsevt. Zh (Kiev), 1961, 16, 35.
6. A. J. Aasen, C. C. J. Culvenor, and L.W. Smith, J. Org. Chem., 1969, 34, 4137.
7. M. R. Roby and F. R. Stermitz, J. Nat. Prod., 1984, 47, 846.
8. E. Roeder, Phytochemistry, 1990, 29, 11.
9. C. C. J. Culvenor and W. G. Woods, Aust. J. Chem., 1965, 18, 1625.
10. H. Rueger and M. H. Benn, Can. J. Chem., 1983, 61, 2526.

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