(+)-3α-HYDROXYLUPANINE, A NEW ALKALOID FROM *Ammopiptanthus mongolicus* (MAXIM.) CHENG F.

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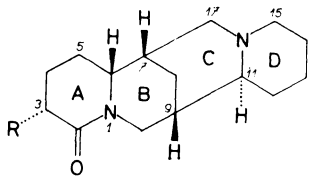
From leaves of *Ammopiptanthus mongolicus* (MAXIM.) CHENG F. were isolated (+)-3α-hydroxylupanine (*I*), (1*R*)-3-O-methyl[1,2,4/3,5,6]-inositol (*V*), salicylic acid, (−)-piptanthine(*III*), 7-hydroxy-4′-methoxyisoflavone (*VIII*), 7,3′-dihydroxy-4′-methoxyisoflavone (*X*) and 4′-methoxyisoflavone-7-β-D-glucopyranoside (*IX*). All the compounds were characterized on the basis of spectral data; the first three compounds were isolated from this material for the first time.

Ammopiptanthus mongolicus (MAXIM.) CHENG F. (*Fabaceae*) is an endemic plant distributed in the Transaltaic, Altaic and Alashanic regions of Gobi in Mongolia. So far, from this plant were isolated quinolizidine alkaloids sparteine, lupanine, isosparteine, piptanthine and piptamine[1], and some isoflavonoids[2]. In our present study of the leaves of *A. mongolicus* we isolated three new compounds. Besides the structural investigation of the new constituents we also confirmed the constitution of the four compounds described, but insufficiently characterized, in previous short communications[1,2].

Chromatography on silica gel of the extract from the leaves of *A. mongolicus* afforded alkaloid *I*, $C_{15}H_{24}N_2O_2$, m.p. 143–145°C, $[\alpha]_D^{20}$ +73·8°. The mass spectrum of *I* exhibited a series of abundant peaks at *m/z* 264 (M^+), 247 (M−17), 246 (M−18), 149, 136 and 134, indicative of a hydroxylupanine skeleton[3]. Comparison of ^{13}C NMR spectra of compound *I* (Table I) with the published data for



I, R = OH
II, R = H

lupanine (*II*), sparteine and their 7-, 8-, 11-, 12- and 13-hydroxy derivatives[4,5] has shown that the hydroxy group is bonded to the ring A. On the basis of the known chemical shifts for lupanine[5] we calculated[6] the shifts for carbon atoms of ring A substituted in position 3, 4 or 5; the best fit of the calculated and observed values was obtained for 3-hydroxylupanine. Analysis of ^{1}H chemical shifts of A-ring protons (Table I), assigned by the heterocorrelated experiment[7], has shown that in deuteriochloroform the protons exhibit a higher order spectrum; a substantially better separation of signals was achieved in perdeuteriobenzene (Fig. 1*a*, Table II). The chemical shifts and coupling constants of the A-ring protons were determined by a 1D COSY experiment[8]. The magnetization transfer was achieved using the H-3 and H-6 protons (Fig. 1*b*, 1*c*). Signals of the H-4a and H-4e or H-5a and H-5e protons are split each by four interactions: this complicates an analysis of antiphase multiplets observed in the 1D COSY spectra. Since in the conventional spectrum the signals of H-4e and H-5a protons are sufficiently separated, the coupling constants were determined by the DISCO method[8,9]. Addition or subtraction of the antiphase and synphase multiplets of the given proton gave simplified multiplets which were "effectively decoupled" relative to the coupling constant used for the magnetization transfer, simultaneously their mutual shift corresponded to this

TABLE I

^{13}C and ^{1}H NMR shifts for (+)-3α-hydroxylupanine (*I*) in $CDCl_3$ (δ, ppm)

Position	^{13}C	^{1}H[a]
2	172·1	—
3	67·6	4·12
4	26·9	1·92 (2 H)
5	22·3	1·78; 1·94
6	59·5	3·40
7	33·4	2·09
8	26·9	1·39; 2·34
9	34·3	1·71
10	47·2	2·61; 4·51
11	63·5	2·08
12	31·5	1·50; 1·61
13	24·4	1·36; 1·75
14	23·6	1·51; 1·71
15	55·0	2·26; 2·90
17	51·3	2·39; 3·07

[a] The ^{1}H NMR shifts were determined from the heterocorrelated experiment; first order approximation; accuracy ±0·02 ppm.

TABLE II

Proton NMR spectral data for the A-ring protons in (+)-3α-hydroxylupanine (*I*) in C_6D_6

Proton	δ, ppm	J, Hz
3	4·17	5·6; 4·5
4a	1·56	12·8; 10·0; 4·5; 3·7
4e	1·85	12·8; 7·5; 5·6; 3·6
5a	1·71	13·4; 10·0; 7·8; 3·6
5e	1·21	13·4; 7·5; 6·7; 3·7
6	2·76	7·8; 6·7; 2·1

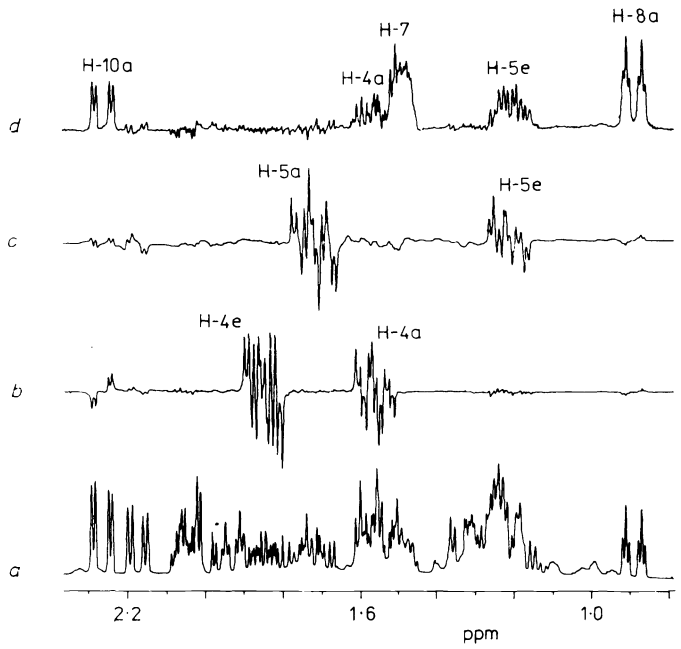


FIG. 1

Proton NMR spectra of (+)-3α-hydroxylupanine (*I*) in C_6D_6: *a* part of conventional spectrum; *b* 1D COSY spectrum with magnetization transfer from H-3 proton (4·17), width of 90°, semi-selective pulse $\tau_{90} = 40$ ms, effective evolution time $\tau_{eff} = 50$ ms; *c* 1D COSY spectrum with magnetization transfer from H-6 proton (2·76); $\tau_{90} = 40$ ms, $\tau_{eff} = 35$ ms; *d* NOE differential spectrum after presaturation of H-6 proton, saturation was achieved by fifty 200 ms pulses. ($\gamma B_2/2\pi = 1{\cdot}6$ Hz) at the individual H-6 resonance frequencies

coupling[9] (Fig. 2). The obtained coupling constants of protons H-3 to H-6 indicated that *1*) the hydroxy group is bonded in the position α and *2*) the ring A exists in a deformed chair conformation. These conclusions agree with a NOE differential experiment[10] (Fig. 1*d*) in which saturation of the H-6 proton resulted in a NOE at δ 2·26 (H-10a, 5%), 1·56 (H-4a, 5%), 1·49 (H-7, 12%), 1·21 (H-5e, 8%) and 0·88 (H-8a, 12%). Saturation of the proton H-3 led to a small NOE only on the H-4a and H-4e protons. On the basis of these data, as well as the presence of the Bohlmann's band in the IR spectrum (2 776 cm^{-1} for compound *I*, 2 761 cm^{-1} for lupanine[11] (*II*)), the compound *I* was assigned the structure (+)-3α-hydroxylupanine.

We further isolated an alkaloid *III*, $C_{20}H_{35}N_3$, m.p. 143–144°C, $[\alpha]_D^{20}$ −24·6. Its mass spectrum exhibited peaks at *m/z* 317 (25%, M^+), 234, 219, 151, 98 and 84; the IR spectrum displayed strong Bohlmann's bands at 2 797 cm^{-1} and 2 758 cm^{-1}. Compound *III* reacted with formaldehyde in formic acid[12] to give the derivative *IV*, $C_{21}H_{35}N_3$. All these data are in accord with those published for (−)-piptanthine[13–15].

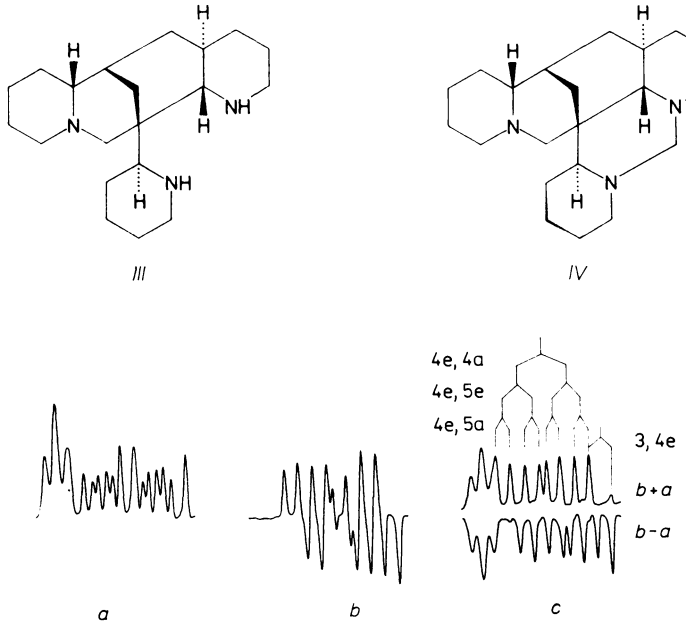
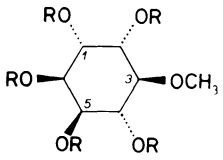


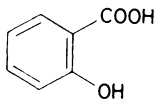
FIG. 2

DISCO analysis of the H-4e proton multiplet in the spectrum of (+)-3α-hydroxylupanine (*I*): *a* synphase multiplet (due to overlapping, also a part of the nearest multiplet is shown); *b* antiphase multiplet (see 1D COSY spectrum on Fig. 1*b*); *c* sum of, or difference between, the spectra *b* and *a*, both the simplified multiplets are mutually shifted for the value of coupling constant *J*(3, 4e)

The residue, obtained after separation of the alkaloids, was repeatedly crystallized from ethanol to give compound *V*, $C_7H_{14}O_6$, m.p. 186–188°C, $[\alpha]_D^{20}$ +60·1°. Its mass spectrum exhibited a very weak molecular ion peak at *m/z* 194 and in the infrared spectrum we found only O—H, C—H and C—O—C bands. The ^{13}C NMR spectrum contained signals of one methoxy group and 6 tertiary carbon atoms bonded to oxygen atoms. Acetylation of *V* with a mixture of acetic anhydride and pyridine afforded the pentaacetate *VI*. In the ^1H NMR spectrum of compound *VI* we observed signals of five acetyl groups, one methoxy group, one proton at δ 3·61 (geminal to the methoxy group) and five protons in the region δ 5·18–5·40; this system was analyzed using the PANIC program. The obtained coupling constants (Table III) are in accord with structure *VI* (3-O-methyl-1,2,4,5,6-pentaacetyl[1,2,4/3,5,6]inositol). The cyclohexane ring of compound *VI* assumes the chair conformation, the acetoxy groups in positions 2, 4, 5 and the methoxy group being equatorial whereas the remaining acetoxy groups (on C-1 and C-6) axial. The isolated compound *V* is thus (1*R*)-3-O-methyl[1,2,4/3,5,6]inositol, identical with (+)-pinitol[16].



V, R = H
VI, R = $COCH_3$



VII

The mother liquors after separation of the inositol *V* were concentrated, the residue was triturated with chloroform and the concentrated chloroform extract was chromatographed on silica gel in chloroform. Crystallization from diethyl ether–hexane afforded compound *VII* which proved to be identical with salicylic acid.

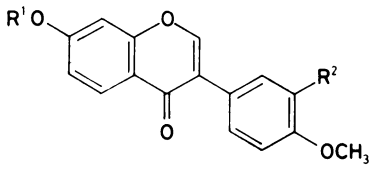
TABLE III

Proton NMR spectral data for 3-O-methyl-1,2,4,5,6-pentaacetyl[1,2,4/3,5,6]inositol (*VI*) in $CFCl_3$

Proton[a]	δ, ppm	Proton[a]	δ, ppm
1	5.30	4	5·35
2	5·19	5	5·20
3	3·61	6	5·32

[a] Coupling constants *J*, Hz: (1,2) = 3·4; (2,3) = 10·3; (3,4) = 9·5; (4,5) = 10·3;(5, 6) = 3·4; (6,1) = 4·8.

The chloroform-insoluble part of the mother liquors was chromatographed on silica gel and crystallization of the individual fractions from ethanol afforded isoflavonoids *VIII*–*X* whose structure was determined mainly by mass and ^{13}C NMR spectroscopy (Table IV). Compound *VIII* was identical with 7-hydroxy-4′-methoxyisoflavone[17]; compound *IX* differed from *VIII* by the presence of a saccharide unit.



VIII, $R^1 = R^2 = H$
IX, $R^1 = C_6H_{11}O_5$; $R^2 = H$
X, $R^1 = H$; $R^2 = OH$

TABLE IV
Chemical shifts (in ppm) in the ^{13}C NMR spectra of isoflavonoids *VIII*–*X* (in CD_3SOCD_3)

Position	*VIII*	*IX*	*X*
2	152·9	153·4	152·9
3	123·2	123·3	123·4
4	174·5	174·6	174·5
4a	116·6	118·4	116·6
5	127·2	126·9	127·2
6	115·1	115·6	115·0
7	162·4	161·4	162·4
8	102·1	103·4	102·0
8a	157·4	156·3	157·3
1′	124·2	123·9	124·7
2′	130·0	129·9	116·4
3′	113·6	113·6	146·0
4′	158·9	159·0	147·4
5′	113·6	113·6	112·1
6′	130·0	129·9	119·7
Glc 1	—	100·0	—
Glc 2	—	73·1	—
Glc 3	—	76·4	—
Glc 4	—	69·7	—
Glc 5	—	77·1	—
Glc 6	—	60·6	—

Comparison of the shifts in the ^{13}C NMR spectrum of *IX* with the known shifts for glycoflavonoids[18] has shown that compound *IX* was 4-methoxyisoflavone-7-β-D--glucopyranoside. The isoflavone *X* contained one hydroxyl more than compound *VIII*; according to mass spectrometry, this hydroxyl is bonded to the ring C. The mutual position of the methoxy and hydroxy groups attached to this ring was determined by ^1H NMR spectra and a 1D NOE differential experiment. Saturation of the methoxy group resulted in NOE on the H-5′ proton (16%); irradiation of the proton H-2 increased intensity of the H-2′ and H-6′ signals. According to these data, compound *X* is 7,3′-dihydroxy-4′-methoxyisoflavone.

EXPERIMENTAL

The plant material from *Ammopiptanthus mongolicus* (MAXIM.) CHENG F. was collected in the Argalanta region of the Southern Gobi in Mongolia. The material was identified by Dr K. Tumbaa of the Mongolian Academy of Sciences, Ulan Bator; its voucher is deposited in the Chemical Institute of the Academy, Ulan Bator.

The melting points were determined on a Kofler block. IR spectra were recorded on a Perkin–Elmer 983 instrument, wavenumbers are given in cm^{-1}. Electron impact mass spectra were measured on a JEOL JMS 100D spectrometer at 70 eV and 300 μA. Proton and ^{13}C NMR spectra were obtained with a Bruker AM 300 instrument at 300 and 75 MHz, respectively. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. The isoflavonoids were analyzed by HPLC on a 150 × 3 mm column packed with Separon SGX C18, 7 μm (Tessek, Prague); mobile phase methanol – 0·5 mol/l sodium acetate in water (50 : 50), flow rate 0·4 ml min^{-1}, detection at 254 nm. Thin-layer chromatography (TLC)was carried out on Silufol UV 254 sheets in the systems S1 chloroform–methanol–diethylamine (90 : 10 : 1), S2 chloroform–methanol (9 : 1), S3 chloroform–methanol–water (14 : 6 : 0·6), detection by UV light at 254 nm or with iodine vapours. Column chromatography was performed on alumina L 40/250 or silica gel L 100/250 (Lachema, Brno).

Isolation of Alkaloids

The dry material (750 g) afforded a mixture of alkaloids (18·8 g) from which sparteine, lupanine and isosparteine were isolated[1]. The residue after this isolation (2·5 g) was separated on an alumina column by gradient elution with toluene–ethanol. The fractions were analyzed by TLC in the system S1. The fractions containing compound of R_F 0·4 were combined, solvents were evaporated and remainder was crystallized from toluene to give 205 mg of alkaloid *I*. Fractions containing compound of R_F 0·30 were crystallized from diethyl ether–hexane (5 : 1) to give 120 mg of base *III*.

(+)-3α-Hydroxylupanine (*I*)

The compound melted at 143–145°C; $[\alpha]_D^{20}$ +73·8° (*c* 1, chloroform). For $C_{15}H_{24}N_2O_2$ (264·4) calculated: 68·15% C, 9·15% H, 10·59% N; found: 68·02% C, 9·18% H, 10·47% N. IR spectrum (chloroform): 3 414 (O–H); 3 133, 2 922, 2 886, 2 851, 2 776 (C–H); 1 645, 1 604 (C=O). Mass spectrum, *m/z* (%): 264 (97), 263 (88), 247 (71), 246 (61), 234 (29), 207 (16), 165 (50), 149 (76), 148 (74), 136 (97), 134 (100), 124 (26), 110 (68), 98 (82), 84 (71). For ^1H and ^{13}C NMR spectra see Tables I and II.

(−)-Piptanthine (*III*)

The alkaloid melted at 143–144°C; $[\alpha]_D^{20}$ −24·6° (*c* 1, chloroform). For $C_{20}H_{35}N_3$ (317·5) calculated: 75·66% C, 11·11% H, 13·23% N; found: 75·59% C, 11·15% H, 13·19% N. IR spectrum (chloroform): 3 277 (N–H); 2 923, 2 843, 2 797, 2 758 (C–H). Mass spectrum, *m/z* (%): 317 (25), 234 (39), 219 (16), 191 (11), 151 (23), 98 (40), 84 (100). Reaction of compound *III* with formaldehyde and formic acid[12] afforded homopiptanthine (*IV*), R_F 0·60 in S1, m.p. 189–190°C. For $C_{21}H_{35}N_3$ (329·5) calculated: 76·55% C, 10·71% H, 12·75% N; found: 76·49% C, 10·53% H, 12·72% N.

(1*R*)-3-O-Methyl[1,2,4/3,5,6]inositol (*V*)

Leaves of *A. mongolicus* (1 kg) were extracted with ethanol (10 l), the extract was filtered, concentrated and the residue dissolved in water. The aqueous solution was extracted successively with chloroform, ethyl acetate and butanol (0·5 l each). After extraction, the aqueous phase was concentrated and the residue crystallized from ethanol to afford 98 mg of compound *V*, m.p. 186–188°C. $[\alpha]_D^{20}$ +60·1° (*c* 1, water). For $C_7H_{14}O_6$ (194·1) calculated: 43·32% C, 7·22% H; found: 43·28% C, 7·30% H. IR spectrum (KBr): 3 480, 3 480, 3 400 (O–H); 2 980, 2 960, 2 870 (C–H). Mass spectrum, *m/z* (%): 158 (1), 144 (3), 103 (10), 102 (11), 87 (100). ^{13}C NMR spectrum (CD_3OD): 84·9, 74·3, 73·8, 73·5, 72·6, 72·0, 60·8.

Salicylic Acid (*VII*)

The chloroform extract, obtained in the isolation of inositol *V* described above, was concentrated and the residue was chromatographed on a column of silica gel in chloroform. The fraction of R_F 0·80 in S3 was concentrated and the residue as crystallized from diethyl ether–hexane (5 : 1) to give 58 mg of compound *VII*, identical (m.p., UV, IR and mass spectra) with an authentic sample of salicylic acid.

Isolation of Isoflavonoids

The ethyl acetate extract, obtained in the isolation of compound *V*, was concentrated and the residue was separated on a column of silica gel (gradient elution with ethyl acetate–ethanol). The fractions were analyzed by HPLC; crystallization of fractions, containing compounds of retention times t_r = 20·0 min, 10·1 min and 6·4 min, afforded isoflavonoids *VIII*, *IX* and *X* which were recrystallized from ethanol.

7-Hydroxy-4′-methoxyisoflavone (*VIII*)

M.p. 265–266°C, for $C_{16}H_{12}O_4$ (268·3) calculated: 71·63% C, 4·51% H; found: 71·55% C, 4·49% H. Mass spectrum, *m/z* (%): 268 (100), 267 (52), 252 (12), 223 (5), 144 (3), 134 (5), 132 (50). ^{1}H NMR (CD_3SOCD_3): 8·29 s, 1 H (H-2); 7·99 d, 1 H (H-5, *J*(5, 6) = 8·7); 7·52 AA′m, 2 H (H-2′ and H-6′, *J*(2′, 3′) = *J*(5′, 6′) = 8·6); 6·99 BB′m, 2 H (H-3′ and H-5′); 6·96 dd, 1 H (H-6, *J*(5, 6) = 8·7; *J*(6, 8) = 2·0); 6·88 d, 1 H (H-8, *J*(8, 6) = 2·0); 3·80 s, 3 H (OCH_3). ^{13}C NMR see Table IV.

4′-Methoxyflavone-7-β-D-glucopyranoside (*IX*)

M.p. 216–217°C, for $C_{22}H_{22}O_9$ (430·4) calculated: 61·39% C, 5·15% H; found: 61·31% C, 5·14% H. $[\alpha]_D^{20}$ −59·1° (*c* 1, methanol). Mass spectrum, *m/z* (%): 268 (100), 267 (15), 252 (12); 223 (5), 144 (2), 132 (10), 117 (8), 99 (9), 60 (22). ^{1}H NMR (CD_3SOCD_3): 8·39 s, 1 H (H-2),

8·05 d, 1 H (H-5, $J(5, 6) = 8·9$); 7·53 AA'm, 2 H (H-2' and H-6', $J(2', 3') = J(5', 6') = 8·8$); 7·24 d, 1 H (H-8, $J(6, 8) = 2·2$); 7·15 dd, 1 H (H-6, $J(5, 6) = 8·9$; $J(6, 8) = 2·2$); 7·00 BB'm, 2 H (H-3' and H-5'); 5·10 d, 1 H (Glc H-1, $J(1, 2) = 7·0$); 3·4–3·9 m, 6 H (Glc H-2, H-3, H-4, H-5 and 2 × H-6); 3·80 s, 3 H (OCH_3). ^{13}C NMR see Table IV.

7,3'-Dihydroxy-4'-methoxyisoflavone (*X*)

M.p. 247–249°C, for $C_{16}H_{12}O_5$ (284·3) calculated: 67·60% C, 4·26% H; found: 67·53% C, 4·30% H. IR spectrum (KBr): 3 416 (O–H); 2 993, 2 926, 2 846 (C–H); 1 620 (C=O). Mass spectrum, *m/z* (%): 284 (100), 283 (10), 270 (10), 269 (30), 241 (13), 213 (14), 137 (14), 112 (15). ^1H NMR (C_5D_5N): 8·46 d, 1 H (H-5, $J(5, 6) = 8·7$); 8·19 s, 1 H (H-2); 7·82 d, 1 H (H-2', $J(2', 6') = 2·2$); 7·34 dd, 1 H (H-6', $J(6', 2') = 2·2$; $J(5', 6') = 8·3$); 7·22 dd, 1 H (H-6, $J(6, 8) = 2·2$); 7·11 d, 1 H (H-8, $J(8, 6) = 2·2$); 7·06 d, 1 H (H-5', $J(5', 6') = 8·3$); 3·80 s, 3 H (OCH_3). ^{13}C NMR, see Table IV.

REFERENCES

1. Selenge D., Telezhanetskaya M. V., Yunusov S. Yu.: Khim. Prirod. Soedin. *1976*, 559.
2. Selenge D., Batsuren D., Batirov E. K., Malikov V. M.: Khim. Prirod. Soedin. *1986*, 242.
3. Cho Y. D., Martin R. O.: Anal. Biochem. *44*, 49 (1971).
4. Bohlmann F., Zeisberg R.: Chem. Ber. *108*, 1043 (1975).
5. Golebiewski W. M., Spenser I. D.: Can. J. Chem. *63*, 716 (1985).
6. Schneider H. J., Hoppen V.: J. Org. Chem. *43*, 3866 (1978).
7. Bax A.: J. Magn. Reson. *53*, 517 (1983).
8. Kessler H., Oschkinat H., Griesinger C.: J. Magn. Reson. *70*, 106 (1986).
9. Kessler H., Müller A., Oschkinat H.: Magn. Reson. Chem. *23*, 844 (1985).
10. Kövér K. E.: J. Magn. Reson. *59*, 485 (1984).
11. Skolík J., Krüger P. J., Wiewiorowski M.: Tetrahedron *24*, 5439 (1968).
12. Valenta Z., Deslongchamps P., Rashid M. H., Wightman R. H., Wilson J. S.: Can. J. Chem. *44*, 2525 (1966).
13. Eisner V., Šorm F.: Collect. Czech. Chem. Commun. *24*, 2348 (1959).
14. Deslongchamps P., Valenta Z., Wilson J. S.: Can. J. Chem. *44*, 2539 (1966).
15. McLean S., Lau P. K., Chang S. K., Murray D. G.: Can. J. Chem. *49*, 1976 (1971).
16. Angyal S. J., MacDonald C. G.: J. Chem. Soc. *1952*, 686.
17. Pelter A., Ward R. S., Gray T. J.: J. Chem. Soc., Perkin Trans. 1, *1976*, 2475.
18. Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J.: Tetrahedron *34*, 1389 (1978).

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