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Structure Determination of Diacholestanes. Their Geochemical Significance.

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Abstract: Six isomeric rearranged cholestanes (diacholestanes) were synthesized and their structures completely determined by X-ray diffraction and NMR. Four of them, the (20*R* + 20*S*) 13β(H),17α(H)- and (20*R* + 20*S*) 13α(H),17β(H)-diacholestanes are identical with the major C-27 geodiacholestanes. The two other isomers, the (20*R*) 13α(H),17α(H)- and 13β(H),17β(H)-diacholestanes, have not yet been found in geological samples.

Diacholestanes are steranes with a rearranged skeleton (Fig. 1). They are currently detected in oils and sediments and are considered to be the reduction products of $\Delta^{13(17)}$-diasterenes themselves formed in the subsurface by clay catalysed backbone reactions of regular sterenes[1, 2]. Although the structures of these diasteranes have not been totally established, they are currently used in petroleum geochemistry as biological markers[3, 4]. Structural approaches to geodiacholestanes have already been realized on hydrogenation products of (20*R*)- or (20*S*)-diacholest-13(17)-enes. Mixtures of varying complexity were obtained (ENSMINGER *et al.*[5],1978 ; PUSTIL'NIKOVA *et al.*[6],1980 ; PEKH *et al.*[7], 1982 ; SIESKIND *et al.*[8], 1991), depending on the reaction conditions and the nature of the catalyst used (PtO_2, Raney nickel). ENSMINGER *et al.*[5] obtained two pairs of diacholestanes from hydrogenation of $\Delta^{13(17)}$-diacholestenes over PtO_2 in acidic medium and proposed (20*R* + 20*S*) 13β(H),17α(H)- and (20*R* + 20*S*) 13α(H),17β(H)-

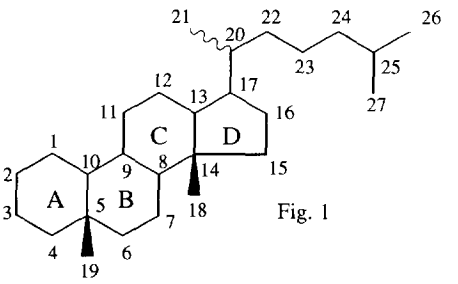


Fig. 1

diacholestane structures, based on analogies with the hydrogenation products of the pentacyclic triterpene hop-17(21)-ene. (Shortened names like 13β(H),17α(H)-, 13α(H)17β(H)- are used in this paper for 5,14-dimethyl-18,19-dinor-8α,9β,10α,13β,17α-, and 5,14-dimethyl-18,19-dinor-8α,9β,10α,13α,17β-cholestanes respectively). Under similar conditions, BAUER *et al.*[9] obtained more complex mixtures ; they synthesized the (20*R* + 20*S*) 13β(H),17α(H) steranes in the C_{27}-C_{29} series from (20*R*)- or (20*S*)-diaster-13(17)-ene-16-ones and deduced the stereochemistry at C-20 by X-ray studies of a reaction intermediate.

We report here the conclusive structure determination of six diacholestanes isolated as individual pure compounds from two diasterane mixtures obtained by hydrogenation of (20*R*)-diacholest-13(17)-ene over PtO_2 and Pd/C respectively.

Hydrogenation of (20*R*)-diacholest-13(17)-ene[10] over 10% Pd/C in ethanol led to the four major steranes I, II, III and IV, in order of elution on DB5 and Supelcowax capillary columns. With PtO_2 as catalyst in acetic acid with a trace of perchloric acid the same compound yielded a different mixture of four major products containing, besides I and II, compounds IIIa and IVa. The major compounds from both reactions were separated into individual components by a sequence of high performance liquid chromatographies (RP18, Cyclobond I, see experimental part). Steranes I and IVa gave monocrystals from acetone-chloroform mixtures and their structures were determined by X-ray diffraction. The other products were oils and their structures were determined by NMR.

X-RAY STRUCTURES OF THE DIACHOLESTANES I AND IVa

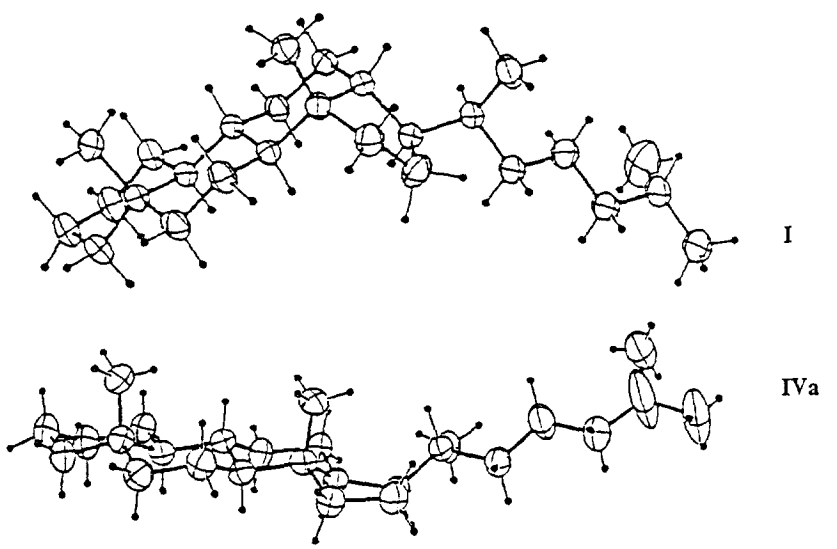


Fig. 2. X-Ray structures of diacholestanes I and IVa.

Figure 2 shows the results of X-ray investigations (published elsewhere[11,12]). It appeared that I and IVa are respectively (20*S*)-13β(H)17α(H)- and (20*R*)-13α(H)17α(H)-diacholestanes (absolute configurations).

STRUCTURE DETERMINATIONS BY ^1H AND ^{13}C-NMR

^1H and ^{13}C NMR investigations corroborated the above results and allowed the structures of II, III IIIa and IV to be defined. After assignment of all δ^1H and $\delta^{13}C$ chemical shifts we proceeded with the stereochemical determinations in order to define the nature of the ring junctions, the orientation of the side chain and the stereochemistry at C-20.

Assignment of chemical shifts : general procedure

The strategy used for signal assignment, described below, was based on scalar ^1H-^1H and ^1H-^{13}C correlations. The dipolar or NOESY correlations were used for stereochemical determinations and for elimination of possible ambiguity.

The C(21)H_3 doublet was the starting point for the signal assignments in the phase sensitive ^1H-^1H COSY. It allowed the location of H-20, which in turn gave H-17. From H-17 it was possible to reach H-16,H-16' and H-13 which in turn led respectively to H-15,H-15' and H-12,H-12'. One of the H-15 protons had a long range coupling (^4J, W) with a methyl group, unambiguously identified as C(18)H_3. The remaining methyl singlet was therefore C(19)H_3. Its δ^1H and $\delta^{13}C$ chemical shifts were practically constant over all of the products investigated and were also identical with those of normal diacholestenes[13, 14]. Further, C(19)H_3 had two long range couplings (^4J, W) with respectively one H-4 and one H-6 proton which in turn gave C(4)H_2 and C(6)H_2, also invariable in the series of diacholestanes. The four spin correlation in which one spin appeared at very high field (about 0.7 ppm) fitted with one methylene and two methine groups and would be attributed either to (H--1,H-1'), H-10 and H-9 or to (H-7,H-7'), H-8 and H-9. The former was correct because C(1)H_2 correlated with C(2)H_2, which in turn correlated with C(3)H_2, whereas the correlation chain of C(7)H_2 is limited to C(6)H_2. The remaining unassigned CH group in the ring system was C(8)H, which led to C(7)H_2 and finally to C(6)H_2 already identified. Finally, the four spin system C(11)H_2-C(12)H_2 could be resolved starting from H-9 or H-13. It was sometimes apparent that in overcrowded regions of the ^1H-^1H COSY map one of the cross peaks of a methylene group could not be resolved. The two chemical shifts were then obtained from the one bond ^1H-^{13}C correlation. The methyls C(26)H_3 and C(27)H_3 correlated with H-25. We also observed that the δ^1H and $\delta^{13}C$ chemical shifts of the nuclei at the end of the side chain were almost the same in each of the diacholestanes. Finally, the chemical shifts of H-22,H-22', H-23,H-23', and of H-24,H-24' were deduced from the corresponding cross peaks in the ^1H-^{13}C correlation map (33-37 ppm, 24-26 ppm and 39.7-39.7 ppm for C-22, C-23 and C-24 respectively).

These assignments were cross-checked with some dipolar correlations. For instance, the geminal protons are characterized by an intense cross peak ; further, 1-4 diaxial interactions between hydrogens or between one hydrogen and one methyl group are readily observed and allow axial-equatorial assignments in methylene groups.

The chemical shifts δ^1H and $\delta^{13}C$ for the different steranes are given in Tables 1 and 1a.

Stereochemical discussions

Nature of ring junctions. Initially we assumed that C(19)H_3 lies on the β face of the molecules. We observed first the constancy of the δ^1H and $\delta^{13}C$ chemical shifts of C(19)H_3 in all the diasteranes investigated. The value $\delta^{13}C = 17.1 \pm 0.2$ ppm is characteristic[15, 16, 17] of a *trans*-decalin pattern for rings A and B and is

Table 1. ^{13}C and ^{1}H Chemical Shifts of Diacholestanes I, II, and IIIa.

Chemical shifts were measured with respect to C_6D_6 and converted to the Me_4Si Scale by : $\delta C = \delta$ meas. +128 ppm, $\delta H = \delta$ meas. + 7.15 ppm.

I. (20*S*)-13β(H),17α(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.42	0.93 β,ax		1.68	15	37.76	1.14 β		1.79
2	27.60	1.18 α,ax		1.78	16	22.75	1.42 β		1.64
3	21.93	1.46 β,ax		1.46	17	43.28		2.14 α	
4	42.80	1.08 α,ax		1.38	18	22.05		0.991	
5	34.07		-----		19	17.19		0.835	
6	42.72	1.10 α,ax		1.40	20	34.34		1.55	
7	22.13	1.37 β,ax		1.46	21	14.41		0.856	
8	45.17		1.02 α		22	37.32	1.24		1.35
9	36.50		1.07 β		23	25.85	1.38		1.38
10	51.21		0.72 α		24	39.73	1.22		1.22
11	25.80	0.94 α,ax		1.63	25	28.34		1.54	
12	23.02	1.54 β,ax		1.65	26	22.87		0.910	
13	49.64		1.37 β		27	22.87		0.912	
14	44.33		-----						

II. (20*R*)-13β(H),17α(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.42	0.94 β,ax		1.70	15	37.66	1.19 β		1.77
2	27.71	1.22 α,ax		1.80	16	25.08	1.42 β		1.66
3	21.96	1.46 β,ax		1.46	17	44.73		2.02 α	
4	42.84	1.10 α,ax		1.40	18	22.00		0.984	
5	34.05		-----		19	17.18		0.835	
6	42.74	1.10 α,ax		1.40	20	36.34		1.49	
7	22.13	1.38 β,ax		1.46	21	19.04		0.993	
8	45.03		0.99 α		22	33.24	1.13		1.46
9	36.38		1.08 β		23	26.18	1.22		1.46
10	51.28		0.73 α		24	39.80	1.21		1.21
11	25.84	0.88 α,ax		1.61	25	28.39		1.54	
12	23.73	1.53 β,ax		1.67	26	22.73		0.912	
13	49.47		1.42 β		27	22.98		0.916	
14	44.95		-----						

IIIa. (20*R*)-13β(H),17β(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.63	0.94 β,ax		1.62	15	39.93	1.37 β		1.65
2	27.79	1.22 α,ax		1.78	16	29.52	1.32 α		1.76
3	22.22	1.48 α,eq		1.57	17	49.19		1.72 β	
4	42.19	1.09 α,ax		1.38	18	25.55		0.933	
5	34.10		-----		19	16.85		0.827	
6	42.19	1.09 α,ax		1.38	20	33.76		1.37	
7	22.31	1.35 α,eq		1.50	21	18.82		0.953	
8	46.08		1.08 α		22	35.90	1.08		1.52
9	31.37		1.05 β		23	24.90	1.28		1.48
10	53.60		0.67 α		24	39.81	1.22		1.22
11	28.51	1.18 β,ax		1.54	25	28.34		1.56	
12	18.08	1.14 α,eq		1.30	26	22.76		0.915	
13	48.71		1.42 β		27	22.93		0.922	
14	44.22		-----						

Table 1a. ^{13}C and ^{1}H Chemical Shifts of Diacholestanes III, IV, and IVa.

Chemical shifts were measured with respect to C_6D_6 and converted to the Me_4Si Scale by : $\delta C = \delta$ meas. +128 ppm, $\delta H = \delta$ meas. + 7.15 ppm.

III. (20*R*)-13α(H),17β(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.84	0.94 β,ax		1.64	15	38.27	1.05 α		1.51
2	27.75	1.21 α,ax		1.78	16	25.41	1.42 α		1.78
3	22.03	1.48 β,ax		1.52	17	46.04		1.69 β	
4	42.88	1.10 α,ax		1.38	18	14.45		0.735	
5	34.27		-----		19	17.33		0.855	
6	42.55	1.18 α,ax		1.41	20	35.73		1.54	
7	23.16	1.38 α,eq		1.50	21	18.92		0.989	
8	55.65		0.80 α		22	33.76	1.14		1.46
9	35.99		1.18 β		23	26.15	1.26		1.46
10	51.35		0.75 α		24	39.82	1.24		1.24
11	31.65	0.70 α,ax		1.84	25	28.38		1.56	
12	25.56	1.18 β,ax		1.72	26	22.77		0.919	
13	52.26		1.07 α		27	22.99		0.935	
14	45.39		-----						

IV. (20*S*)-13α(H),17β(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.88	0.94 β,ax		1.64	15	38.38	1.05 α		1.54
2	27.79	1.21 α,ax		1.78	16	22.81	1.44 α		1.65
3	22.05	1.48 α,ax		1.52	17	44.69		1.80 β	
4	42.91	1.12 α,ax		1.38	18	14.37		0.735	
5	34.31		-----		19	17.37		0.856	
6	42.58	1.18 α,ax		1.41	20	33.70		1.63	
7	23.20	1.38 α,eq		1.52	21	14.71		0.876	
8	55.68		0.79 α		22	37.21	1.23		1.36
9	36.13		1.19 β		23	25.87	1.37		1.37
10	51.39		0.75 α		24	39.81	1.23		1.23
11	31.66	0.68 α,ax		1.86	25	28.39		1.55	
12	24.47	1.14 β,ax		1.64	26	22.91		0.929	
13	52.30		1.04 α		27	22.91		0.929	
14	45.04		-----						

IVa. (20*R*)-13α(H),17α(H).

C	$\delta^{13}C$	$\delta^{1}H$			C	$\delta^{13}C$	$\delta^{1}H$		
1	24.81	0.94 β,ax		1.66	15	39.65	1.02 α		1.59
2	27.72	1.21 α,ax		1.77	16	28.74	1.58 β		1.89
3	22.01	1.50 β,ax		1.50	17	46.27		1.82 α	
4	42.80	1.09 α,ax		1.37	18	14.73		0.767	
5	34.17		-----		19	17.28		0.845	
6	42.48	1.18 α,ax		1.41	20	37.14		1.57	
7	23.05	1.38 α,eq		1.50	21	20.63		1.025	
8	56.11		0.74 α		22	36.29	1.09		1.50
9	35.55		1.135β		23	24.51	1.22		1.43
10	51.27		0.74 α		24	39.94	1.21		1.21
11	32.70	0.68 α,ax		1.92	25	28.38		1.55	
12	25.42	1.41 β,ax		1.84	26	22.78		0.921	
13	53.70		1.41 α		27	22.99		0.925	
14	45.01		-----						

also found in rearranged steroids[11, 12]. Also the chemical shifts of all nuclei in ring A, as well as those of C-6 and C-7 in ring B are comparable with the sole exception of C-10 in diasterane IIIa, indicating that the A/B ring junction is *trans* in the series.

Another general observation was the existence of NOE interactions between C(19)H_3, C(18)H_3 and H-9, which are therefore all located on the β face of the molecule. Further, the coupling ^{3}J(H-9,H-10) and ^{3}J(H-8,H-9), about 10.5 Hz, indicated the *trans* nature of ring junction B/C. This result was corroborated by the dipolar correlation between H-10 and H-8, which was observed when the chemical shift difference between these nuclei was significant.

The junction between rings C and D was deduced from the ^{13}C chemical shift of C(18)H_3. It is in the range δ = 22-25 ppm for I, II and IIIa, whereas it covers the range δ = 14.4-14.7 ppm for III, IV and IVa. This difference corresponds to that observed for angular methyl groups in *cis* and *trans* hydrindane moieties[18], showing that the C/D junction is *cis* (H-13, β) in compounds I, II and IIIa and *trans* (H-13, α) for III, IV and IVa.

Orientation of side chain. The existence of an Overhauser effect between C(18)H_3 and H-17 demonstrates that the orientation of the side chain is α (H-17, β) for steranes III, IIIa and IV. This interaction was not observed for compounds I, II and IVa, indicating that the side chain is probably β (H-17, α). Corroboration was obtained on analysis of (Fig. 3) the coupling pattern of H-17 in order to measure the value of ^{3}J(H-13,H-17). The measured value ^{3}J = 10.5 Hz for I, II, III and IV indicates a 13β(H),17α(H) arrangement for I and II, and a 13α(H),17β(H) arrangement for III and IV. Similarly the value ^{3}J(H-13,H-17) = 4 Hz found for IIIa indicates a 13β(H),17β(H) structure. In the case of IVa, however, the COSY cross peak of H-13 and H-17 and that of H-12,H-12' overlapped, and prevented us from measuring ^{3}J(H-13,H-17). Fortunately the β orientation of the side chain was known from the X-ray studies.

Stereochemistry at C-20. The Overhauser effects of C(21)H_3 and the value of the coupling ^{3}J(H-17,H-20) are both a function of the dihedral angle defined by H-17, C-17, C-20 and H-20. If these data are available for each member of a pair of diastereomers, the conformation and the configuration of each isomer are unequivocally established. A similar method has recently been proposed for pregnanes having polar substituents on C-20[19].

Figure 3 illustrates the different methods we used to obtain ^{3}J(H-17,H-20). The value of ^{3}J(H-17,H-20) and the NOEs of C(21)H_3 for each sterane are given in Table 2. The stereochemistry at C-20 was established, as below, with the aid of a set of Newman projections along the (C-17,C-20) bond for three of the four possible pairs of diasteranes (Fig. 4).

In sterane II, which is 13β(H),17α(H), C(21)H_3 has NOE effects with (H-17, α) and (H-16, β). As the coupling ^{3}J(H-17,H-20) is small (5 Hz), H-17 and H-20 are *gauche*. We now had to choose (Fig. 4), among the six possible 13β(H),17α(H)-diacholestanes, the isomers which was compatible with the experimental value of ^{3}J(H-17,H-20) and with the observed polar correlations of C(21)H_3. It appeared that the unique structural possibility for sterane II is 20*R* with a *gauche+* preferred conformation. Sterane I has the 20*S* configuration as already established by X-rays. Its preferred conformation is also *gauche+* because of the proximity in space of C(21)H_3, (H-13, β) and (H-16,β) showing that the conformations in C_6D_6 solution and in the solid state are the same (Fig. 2).

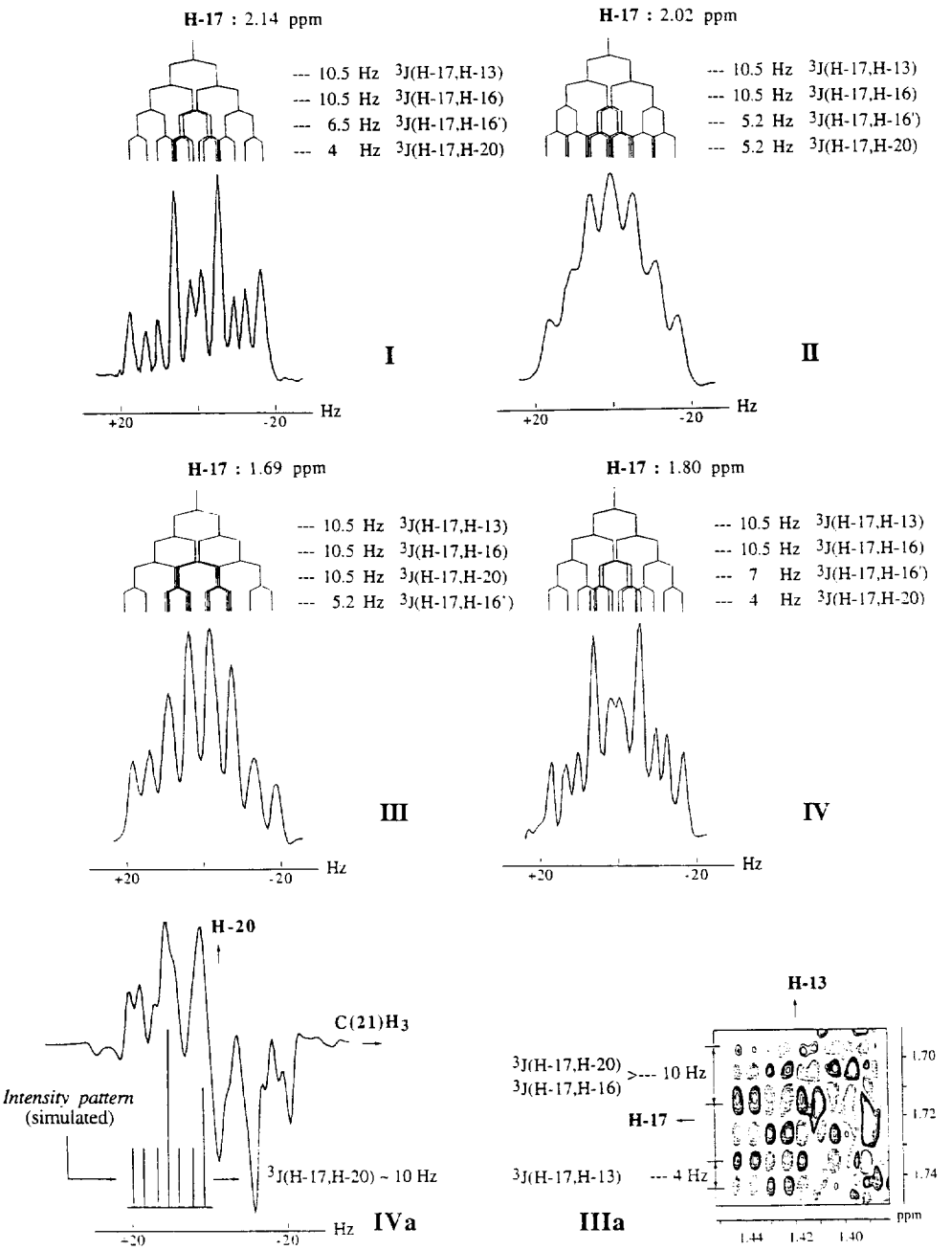


Fig. 3 : Determination of ^3J(H-17,H-13) and ^3J(H-17,H-20) of the diacholestanes :

For **I** and **II** from the fine structure of H-17 (proton spectra) ; for **III** and **IV** from the fine structure of H-17 (NOESY cross peak of C(18)H_3 with H-17) ; for **IIIa** from the COSY cross peak of H-13 with H-17 ; for **IVa**, only ^3J(H-17,H-20), from the COSY cross peak of H-20 with C(21)H_3. The coupling ^3J(H-17,H-20) is deduced from the simulated cross peak intensity pattern. For the simulation : ^3J[H-20,C(21)H_3] = 6 Hz ; ^3J(H-20,H-17) = ^3J(H-20,H-22) = 9 Hz ; ^3J(H-20,H-22') = 3 Hz.

The values given for ^3J(H-17,H-16) or ^3J(H-17,H-16') had been measured on the corresponding cross peaks from the COSY map.

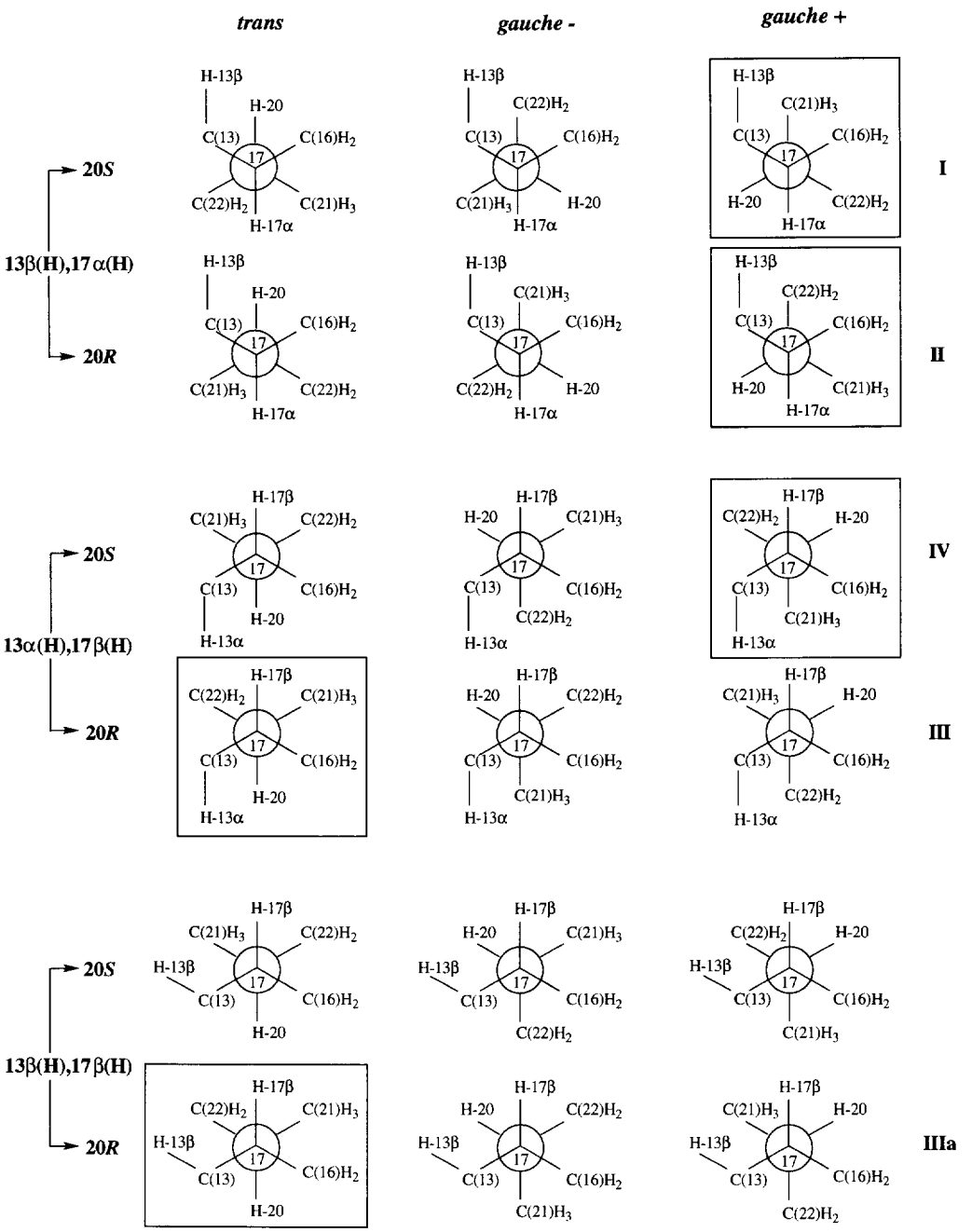


Fig. 4. Set of Newman projections along the (C-17,C-20) bond of the various diacholestanes.

Possible environments of $C(21)H_3$ in *trans*, *gauche*+ and *gauche*- conformers of 13β(H),17α(H)-, 13α(H),17β(H)- and 13β(H),17β(H)-diacholestanes in the 20*R* and 20*S* series. In the boxes are shown the Newman projections corresponding to those conformations and configurations in agreement with the observed NOEs and ^3J(H-17,H-20).

Table 2. Summary of the NMR Investigations .

Junction of the Rings A, B, C and D

	I	**II**	**IIIa**	**III**	**IV**	**IVa**
$\delta^{13}C(19)H_3$ (ppm) *	17.19	17.18	16.85	17.37	17.33	17.28
Junction A/B	*trans*	*trans*	*trans*	*trans*	*trans*	*trans*
^3J(H-9,H-10) (Hz)	10.5	10.5	10.5β	10.5	10.5	10.5
H-9 **	β	β	β	β	β	β
^3J(H-9,H-8) (Hz)	10.5	10.5	10.5	10.5	10.5	10.5
H-8	α	α	α	α	α	α
Junction B/C	*trans*	*trans*	*trans*	*trans*	*trans*	*trans*
$\delta^{13}C(18)H_3$ (ppm)	22.50	22.00	25.55	14.34	14.50	14.73
Junction C/D	*cis*	*cis*	*cis*	*trans*	*trans*	*trans*
H-13	β	β	β	α	α	α

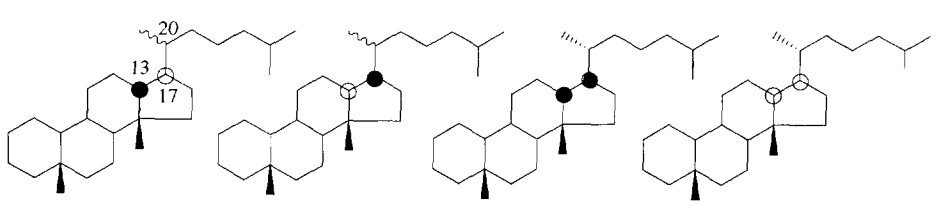
* $C(19)H_3$ is supposed to be β. ** $C(18)H_3$ is β because of its NOE with (H-9,β).

Orientation of the Side Chain

	I	**II**	**IIIa**	**III**	**IV**	**IVa**
^3J(H-13,H-17) (Hz)	10.5	10.5	4	10.5	10.5	measure imposs.
NOEs [$C(18)H_3$,H-17]	-	-	+	+	+	-
H-17	α	α	β	β	β	α (from X-rays)

Stereochemistry at C-20

	13β(H),17α(H)	13β(H),17α(H	13β(H),17β(H)	13α(H),17β(H	13α(H),17β(H)	13α(H),17α(H)
	I	**II**	**IIIa**	**III**	**IV**	**IVa**
NOEs of $C(21)H_3$	H-13β;H-16β	H-17α;H-16α	H-17β;H-16β	H-17β;H-16β	H-13α;H-16α	H-12α;H-17α
^3J(H-17,H-20) (Hz)	4 Hz	5 Hz	10 Hz	10.5 Hz	4 Hz	10 Hz
Configuration at C-20	**20*S***	**20*R***	**20*R***	**20*R***	**20*S***	**20*R***
Preferred conformation	*gauche* +	*gauche* +	*trans*	*trans*	*gauche* +	*trans*



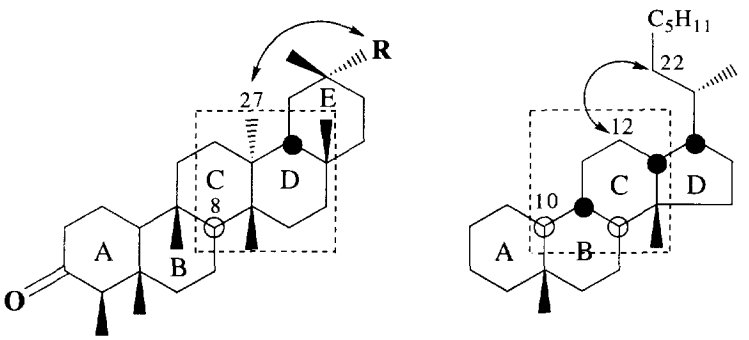
13β(H), 17α(H) **I** (20*S*)+ **II** (20*R*) | 13α(H), 17β(H) **III** (20*R*)+**IV** (20*S*) | 13β(H), 17β(H) **IIIa** (20*R*) | 13α(H), 17α(H) **IVa** (20*R*)

Similarly, we showed that III and IV, both 13α(H),17β(H), are respectively 20*R* and 20*S* . Indeed, in sterane III, ^3J(H-17,H-20) = 10.5 Hz indicating that H-17 and H-20 are *trans*. Because of the polar interaction of C(21)H_3 with (H-16, β) and (H-17, β) the configuration is 20*R*, as seen in figure 4. Consequently, sterane IV must be the 20*S* isomer of III and its preferred configuration is *gauche+* due to the observed Overhauser effects with (H-13, α) and (H-16, α). The proximity in space of C(22)H_2 and (H-12,α), only possible in the 20*S* configuration, corroborates the above conclusions.

The stereochemistry of IIIa and IVa, respectively 13β(H),17β(H) and 13α(H),17α(H), could also be established without ambiguity, although we possessed only one member of each pair of the diastereomers at C-20. In sterane IIIa, C(21)H_3 showed an Overhauser effect with (H-16, β) and (H-17, β), H-17 and H-20 being *trans*. Comparing the two *trans* configurations of the 13β(H),17β(H) isomers in figure 4, it appeared that sterane IIIa is undoubtedly 20*R*. Protons H-17 and H-20 are also *trans* in sterane IVa, and C(21)H_3 has an important Overhauser effect with (H-12,α) and a smaller interaction with (H-17, α). These conditions are only fulfilled in the 20*R* configuration as seen on molecular models. Here also the preferred conformation is the same in C_6D_6 solution as in the solid state (see Fig. 2).

The results of the NMR sections are summarized in Table 2.

The structure of diacholestane IIIa in which H-17 and H-20 are *trans* showed some interesting particularities needing further comments. Indeed the *trans* conformation of H-17 and H-20 generates 1-5 interactions between C(22)H_2 and C(12)H_2 which could be minimized if ring C is converted from the chair into a boat conformation. Indeed the chemical shift of C(18)H_3 had an unusual high value (25.5 ppm), reflecting



R : CH_3 Friedeline
R : CO_2H Pulponic acid

Diacholestane IIIa

Fig. 5. Analogy of the situation of C-8 and ring D in friedelin with that of C-10 and ring C in diacholestane IIIa.

modifications in the γ interactions of C(18)H_2 and C(12)H_2. On the other hand, the chemical shift of all carbon atoms in ring C exclusively varied by more than 0.1 ppm between 293°K and 323°K. These two observations favoured a flexible conformation of ring C in diasterane IIIa. Furthermore, we already noted that the chemical shift for C-10 in sterane IIIa (53.6 ppm) differed by about 2.5 ppm from the corresponding values in the other rearranged steranes (51.2 - 51.4 ppm). The situation for C-10 with respect to ring C in the steranes may be

compared (Fig. 5) to that of friedelin (R : CH3) and pulponic acid (R : CO_2H). Due to steric crowding between $C(27)H_3$ and R the D and E rings in friedelin have a boat conformation[20], whereas in pulponic acid (R = CO_2H, less bulky) these rings are in a chair form[21]. The chemical shift of C-8 is equal to 53.1 ppm in friedelin, but only of 50.7 ppm in pulponic acid [21, 22], the higher value of δ^{13}C-8 in friedelin resulting from the boat conformation of ring D. Most likely, ring C in sterane IIIa is also in a boat conformation because of the higher value of the chemical shift of C-10.

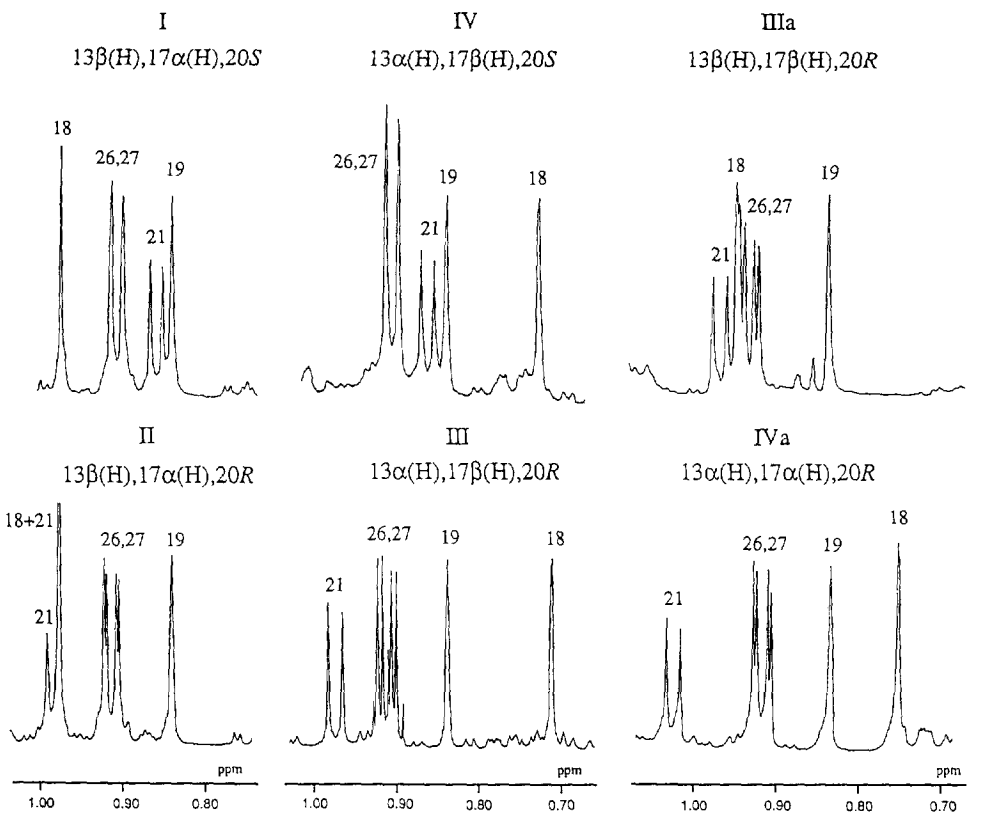


Fig. 6. High field part of the 400 MHz ^{1}H-spectra of the diacholestanes I, II, III, IIIa, IV and IVa in C_6D_6 showing therelationship of the chemical shifts of $C(18)H_3$ and $C(21)H_3$ with the stereochemistry of the molecule.

The *structures having been established*, it appeared that useful information would be directly available from the one dimensional ^{13}C and ^{1}H-NMR spectra. We had already seen that the ^{13}C resonances of $C(19)H_3$ and $C(18)H_3$ gave the nature of the A/B and C/D ring junctions. The ^{13}C resonance of $C(21)H_3$, about 19 ppm in the 20*R* isomers, is shifted upfield by 4.45 + 0.9 ppm when the configuration at C-20 becomes 20*S*. The proton spectra also give indications about the C/D junction and the configuration at C-20 (Fig. 6). Indeed, $C(18)H_3$ is

the most shielded methyl signal when the C/D rings are *trans* -linked and in the 20*R* isomers the most deshielded methyl signal is that of $C(21)H_3$.

GEOCHEMICAL CONSIDERATIONS

Several characteristic structural features can be deduced from the mass spectra of the diacholestanes and may be useful for their recognition in complex geochemical mixtures. They all possess the same fragment ions (Table 3, Fig. 7) which are generally characteristic of rearranged cholestanes : e.g. m/z = 189 ; 259 (M^+ - side chain) ; 217 (base peak) and 372 (M^+). Identical mass spectra were obtained for the pairs of diasteranes (I-II)

Table 3. Mass spectral Data (EI, 70 eV), giving the relative Intensities of the main Fragments of the six Diacholestanes I-IVa.

m/z	**372**	**357**	**344**	**343**	**315**	**287**	**259**	**243**	**232**
I, II	52	11	-	-	4	20	53	8	4
III, IV	49	19	<1	-	4	4	30	10	35
IIIa	19	4	11	-	<1	<1	65	4	20
IVa	62	2	-	-	<1	<1	46	5	27
m/z	**217**	**203**	**189**	**177**	**163**	**149**	**135**	**121**	**109**
I, II	100	15	54	21	35	43	27	20	35
III, IV	100	13	75	18	44	37	26	17	32
IIIa	100	19	11	21	32	42	35	28	47
IVa	100	4	84	15	33	24	18	14	23

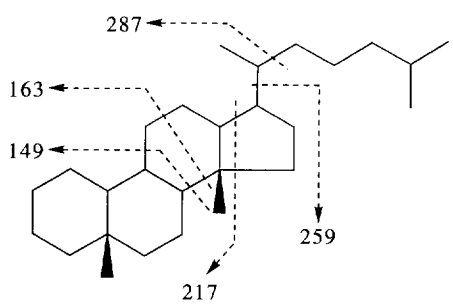


Fig. 7. Main fragment ions observed in the mass spectra of the various diacholestanes (M^+= 372).

and (III-IV) respectively. It can be seen that the mass ratio **R** = 149/163 can be correlated with the stereochemistry of the C/D ring junction. Indeed **R**<1 for compounds III, IV and IVa where the junction is *trans* and concomitantly an enhanced m/z = 232 fragment is observed. For steranes I, II and IIIa possessing the *cis* C/D ring junction **R**>1 and the m/z = 232 fragment is significantly smaller (especially in the case of I and II).

Furthermore, the compounds with the C/D *cis* junction display a slightly enhanced m/z = 259 fragment. Moreover, the spectrum of the 13β(H),17β(H) sterane IIIa is easily recognizable because of its particularly low m/z = 189 peak and the presence of an unusual m/z = 344 (M^+ -28) fragment.

From the GC-MS-MS investigations of previously-studied sediments (Creveney, Jouy-aux-Arches and Semecourt, Paris Basin, Toarcian) we have shown that the first eluting and major pair of geodiacholestanes is identical to I and II, the 20*S* isomer eluting first. These results are in agreement with those of BAUER et al.[9] and the hypothesis of ENSMINGER *et al.*[5]. The second and minor pair of geodiacholestanes has now been conclusively identified as the 13α(H),17β(H)-diacholestanes (III and IV) with a reversed elution order for the C-20 isomers, by analogy with the observations made on 5α(H),14β(H) regular cholestanes (MOLDOWAN *et al.*[23], 1980). The 13β(H),17β(H) and 13α(H),17α(H) isomers have not been detected in geological samples so far. This may be explained by their lower stability and their particular ease of isomerisation on silicoaluminates, as shown by preliminary simulation experiments.

EXPERIMENTAL

HPLC separations

HPLC separations were performed on a WATERS 6000A instrument equipped with a R-400 refractometer as detector. Columns used : a) *RP18 Zorbax* ODS (25 cm x 2.5 cm, 0,2μ film thickness) for gross separations; solvent system : acetone with 5-10 % water, depending upon the mixtures ; flow rate : 10-15 ml/min.- b) *Cyclobond-I* (25 cm x 0.25 cm) for refined separations leading to pure individual products.; solvent system : acetonitrile with 5-10 % ethylacetate, depending upon the mixtures ; flow rate : 0.5 ml/min.

GC-MS data

GC-MS data were obtained at 70 eV on a GC-MS FINNIGAN TSQ 70 spectrometer equipped with capillary columns (DB5 and Supelcowax, 60 m x 0.25 mm, 0.25 μm film thickness). Chromatographic conditions : *DB5* : 60°C (1 min isoth.), 60-300°C (3°/min), 300°C (15 min isoth.). *Supelcowax* : 60°C (1 min isoth.), 60-280°C (3°/min), 280 °C (30 min isoth). He (carrier gas) : 1 ml/min in both cases.

Table 4. Relative GC Retention Times of the Diacholestanes I-IVa on two different Phases.
(Cholestane = internal Standard)

	I	**II**	**III**	**IIIa**	**IV**	**IVa**	Cholestane
DB5	0.935	0.947	0.955	0.955	0.961	0.995	1
Supelcowax	0.906	0.918	0.933	0.934	0.938	0.989	1

^1H and ^{13}C-NMR

^1H and ^{13}C-NMR experiments were performed on a BRUKER AM400 and a BRUKER AM500X spectrometer working at 400.1 MHz and 500.1 MHz for protons and 100.6 MHz and 125.75 MHz for ^{13}C respectively. According to the samples, solutions were made from 0.35 ml of C_6D_6 and 2-10 mg of steranes. Spectra were generally recorded at room temperature (about 293 °K) on the AM400, without stabilisation. For

inverse detected ^{13}C-^{1}H correlations and for all experiments on the AM500X the temperature was stabilised at 298 °K. For the NOESY experiments samples were degassed either by preparing sealed tubes after several freeze-pump-thaw cycles or by bubbling argon through the solution and fitting a teflon serum cap. No significant difference was observed between these two procedures. The 2D experiments were acquired and processed with the software provided by BRUKER on ASPECT 1000 or on ASPECT X32. Typical acquisition and processing conditions for COSY and NOESY experiments were : relaxation delay of 1 to 2 seconds, 512 t_1 increments ; 1024 to 2048 t_2 points ; sweep width of 2 ppm. Sine bell squared and shifted ($\pi/4$, $\pi/6$ and $\pi/8$) apodization functions were used for processing. The mixing time in the NOESY experiments, generally set at 1.2-1.5 seconds, was also varied between 0.8 and 2 seconds, without substantial change in the results. For ^{1}H-^{13}C (^{13}C detected) and ^{13}C-^{1}H (^{1}H detected) correlations, we used the same relaxation delays, 256 to 512 t_1 increments, 1024 to 2048 t_2 points, the sweep width being respectivley 2 ppm for ^{1}H and 60 ppm for ^{13}C. Lorentz and Gaussian deconvolution were generally used in the processing. The number of scans was set for an overall acquisition time of about 12 to 16 hs.

Melting points

Sterane I : 83.5 °C ; Sterane IV : 85.0 °C.

REFERENCES

1. Rubinstein, J.; Sieskind, O.; Albrecht, P. *J. Chem. Soc. Perkin I* **1975**, 1833-1836.
2. Sieskind, O.; Joly, G.; Albrecht, P. *Geochim. Cosmochim. Acta* **1979**, *43*, 1675-1679.
3. Mackenzie, A.S.; Brassell, S.C.; Eglinton, G.; Maxwell, G.R. *Science*, **1982**, *217*, 491-504.
4. Kenneth, E.P.; Moldowan, J.M. *The Biomarker Guide : Interpreting Molecular Fossils in Petroleum and Ancient Sediments*; Prentice Hall, Englewood Cliffs: New Jersey. 1993; pp 190-145.
5. Ensminger, A.; Joly, G.; Albrecht, P. *Tetrahedron Lett.* **1978**, *18*, 1575-1578.
6. Pustil'nikova, S.D.; Abryutina, N.N.; Kayukova, G.P.; Petrov, A.A. *Neftekhimiya* , **1980**, *20*, 183-193 (Chem. Abstr. *93*, 168482c).
7. Pekh, T.; Pustil'nikova, S.D.; Abryutina, N.N.; Kayukova, G.P.; Petrov, A.A. *Neftekhimiya* **1982**, *22*, 21-29.
8. Sieskind, O.; Kintzinger, J.P.; Metz, B.; Albrecht, P. Structural elucidation of diacholestanes. In *Organic Geochemistry : Advances and Applications in the Natural Environment*. 15 th. International Meeting on Organic Geochemistry. Poster Abstracts; Manning, D.D.A., Ed.; Manchester University Press, 1991 ; pp 206-207.
9. Bauer, P.E.; Nelson, D.A.; Watt, D.D.; Reibenspiess, J.H.; Anderson, O.P.; Seifert, W.K.; Moldowan, J.M. *J. Org. Chem.* **1985**, *50*, 5460-5465.
10. Sieskind, O.; Albrecht, P. *Tetrahedron Lett.* **1985**, *26*, 2135-2137.
11. Metz, B.; Louis, R.; Sieskind, O.; Albrecht, P. **1** (submitted to *Acta Crystallographica*).
12. Metz, B.; Louis, R.; Sieskind, O.; Albrecht, P. **2**.(submitted to *Acta Crystallographica*).
13. Sieskind, O.; Kintzinger, J.P.; Albrecht, P. *J. Chem. Soc. Chem. Comm.* **1989**, 133-135.
14. Tal, M.D.; Gottlieb, H.E.; Ben-Ari, C.; Mazur, Y. *Tetrahedron* **1981**, *37*, 4331-4335.
15. Dalling, D.K.; Grant, M.G.; Paul, E.G. *J. Amer. Chem. Soc.* **1973**, *95*, 3718-3724.
16. Dalling, D.G.; Grant, M.G. *J. Chem. Soc.* **1974**, *96*, 1827-1834.
17. Beierbeck, H.; Saunders, J.K.; Apsimon, J.W. *Can. J. Chem.* **1977**, *55*, 2813-2828.
18. Gramain, J.C.; Quirion, J.C. *Magn. Res. Chem.* **1986**, *24*, 938-946.
19. Marat, K.; Templeton, J.F.; Ling, Y. *Magn. Res. Chem.* **1993**, *31*, 17-22.
20. Radler De Aquino Neto, F.; Sanders, K.M. *J. Chem. Soc. Perkin Trans. I* **1983**, 181-183.
21. Ramaiah, P.A.; Devi, P.U.; Frolow, F.; Lavie, D. *Phytochemistry* **1984**, *23*, 2251-2255.
22. Gottlieb, H.E.; Ramaiah, P.A.; Lavie, D. *Magn. Res. Chem.* **1985**, *23*, 616-620.
23. Moldowan, J.M.; Seifert, W.K.; Haley, M.G.; Djerassi, C. *Geochim. Cosmochim. Acta* **1980**, *44*, 1613.

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