Tetrahedron Vol 45, No. 14, pp 4457 to 4470, 1989
Printed in Great Britain

0040-4020/89 $3 00+.00

IDENTIFICATION OF DES-*A*-TRITERPENOID HYDROCARBONS OCCURRING IN SURFACE SEDIMENTS

J.M. TRENDEL, F. LOHMANN, J.P. KINTZINGER and P. ALBRECHT*

Département de Chimie, Université Louis Pasteur
1 rue Blaise Pascal, 67008 Strasbourg, France

A. CHIARONI, C. RICHE, M. CESARIO, J. GUILHEM and C. PASCARD

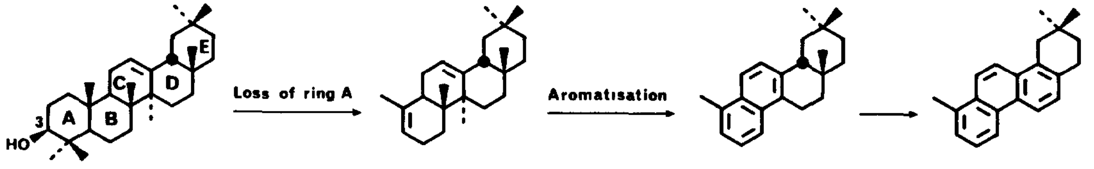
Institut de Chimie des Substances Naturelles, C.N.R.S.
91190 Gif-sur-Yvette, France

(*Received in Belgium* 10 *April* 1989)

Abstract : Molecular structures of eight tetracyclic hydrocarbons occurring in sediments were established either by synthesis of the corresponding reference compounds or spectroscopic studies (MS, NMR, X-ray diffraction) of the natural products isolated from a pond mud. These products are derived from higher plant 3-oxygenated pentacyclic triterpenes (lupane, oleanane and ursane series) by microbially mediated loss of ring A and subsequent aromatisations.

INTRODUCTION

Polycyclic hydrocarbons in the form of alkanes, alkenes and hydroaromatic structures related to 3-oxygenated pentacyclic triterpenes occurring in higher plants have been observed in many geological samples, including petroleums, recent and ancient sediments, as well as immature coals[1-5]. These molecular markers are generally considered as indicators of a terrigenous contribution to the geological organic matter and give useful informations on the chemical processes occurring in sediments. Thus, the presence of the tetracyclic aromatic hydrocarbons **3** and **4** in recent or ancient sediments points towards a transformation pathway of higher plant precursor triterpenes which apparently proceeds by loss of ring A followed by progressive aromatisation from B to D[2,3] (fig. 1). Tentative or full identification in recent sediments of olefinic compounds such as **2** or **11**[4,5] (fig. 1 and 2), which may be considered as potential intermediates, further strengthens this hypothetical scheme of degradation.



1 2 3 4

Figure 1 : Hypothetical degradation scheme of olean-12-en-3β-ol (β-amyrin) in surface sediment.

The occurrence of significant amounts of these tetracyclic hydrocarbons in surface sediments not older than a few decades strongly suggested that microbiological processes were responsible for their formation[2,5,6]. These considerations led us to carry out incubation experiments with two radiolabelled triterpenes, lupan-3-one and olean-3β-ol (β-amyrin) in the bottom mud of a pond near Huttenheim (Alsace, France). The results of these experiments, which will be reported elsewhere, clearly confirmed the above mentioned hypothesis. They were dependent upon an unambiguous recognition of radioactive transformation products which requires the prior precise characterisation of the tetracyclic components naturally occurring in the mud of the pond. We would like to report here the identification of eight of these compounds belonging to the lupane, oleanane and ursane series.

RESULTS AND DISCUSSION

As reported in previous studies[7], the investigated sediment shows a high organic carbon level (17%) due to an important higher plant input and to the anoxic conditions prevailing in the mud. The total organic extract was analysed as described in the experimental part and several compounds related to different triterpene series were found in the hydrocarbon fractions.

Lupane series

Three compounds belonging to the lupane series were detected in the sediment.

Two of them, **7** and **11**, have been previously reported in deltaic sediments[5]. Their identification was established by comparison of their analytical data with those of the corresponding synthetic compounds (fig. 2), based on the following criteria : identical mass spectra (obtained by GC-MS), co-elution on two different phases in high resolution gas chromatography. The third component, which was isolated from the sediment, displayed NMR and MS data consistent with structure **12**. A monocrystal of this compound was further obtained by careful recrystallisation from a mixture of methanol and dichloromethane and submitted to X-ray diffraction, which indeed confirmed our structural hypothesis (fig. 3). Figure 2 shows the scheme adopted for the preparation of reference compounds. (10βH)-Des-*A*-lupan-5-one **6** was obtained from lupan-3β-ol **5** *via* a described sequence[8,9]. Wolff-Kishner reduction of ketone **6** gave rise to a mixture (95:5) of (10βH)-des-*A*-lupane **7** and des-*A*-lupane **8** separable by reverse phase HPLC. Treatment of ketone **6** by lithium aluminium hydride afforded the two alcohols **9** and **10**. Dehydration of the latter with phosphorus oxychloride/pyridine furnished des-*A*-lup-5(10)-ene **11** and another isomer readily separable on silver nitrate impregnated silica gel. This isomer, which surprisingly represented 40% of the mixture, displayed spectral characteristics identical with des-*A*-lup-9-ene **12** (F, ^{1}H-NMR, MS). Absolute configuration of the isolated product **12** was established as being the same as the synthetic lupane derivative from $[\alpha]_D$ measurements.

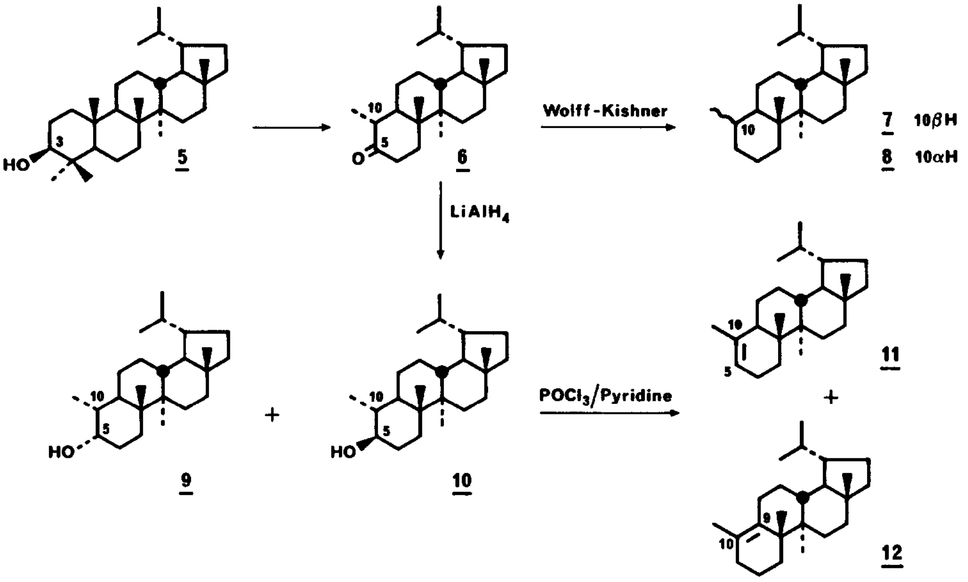


Figure 2 : Synthetic scheme for the preparation of reference hydrocarbons 7, 11 and 12 in the lupane series.

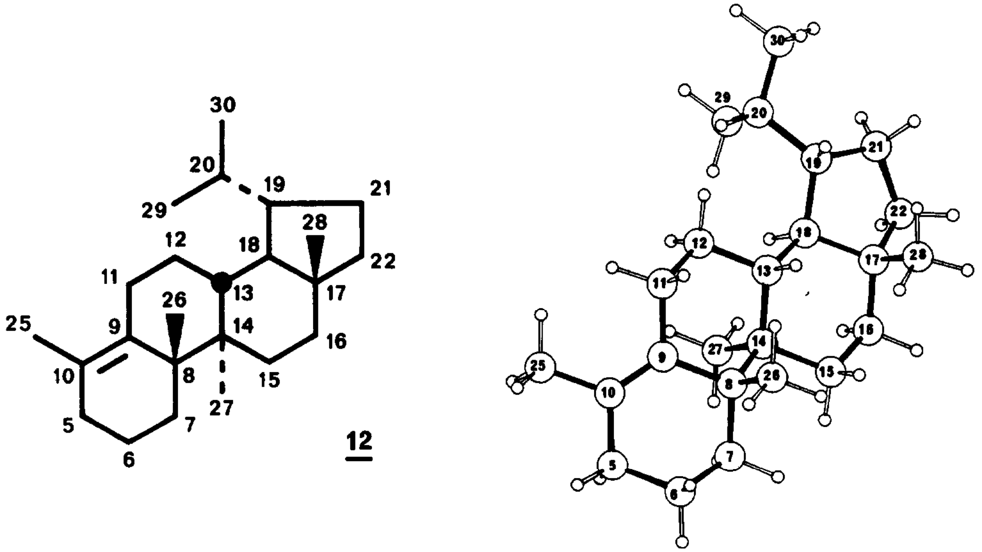


Figure 3 : Carbon numbering and perspective drawing of structure 12 obtained from X-ray crystallography.

Oleanane series

Two hydrocarbons, **2** and **3**, were isolated from the mud.

Structural elucidation of des-*A*-oleana-5(10),12-diene **2** was supported by MS and NMR data. Indeed the mass spectrum of **2** exhibits a molecular ion at *m/z* = 326 corresponding to an elemental composition of $C_{24}H_{38}$ and diagnostic fragment ions at *m/z* = 108 (base peak) and 218. These two even-numbered ions suggest a fragmentation pattern similar to olean-12-ene derivatives, formally corresponding to a retro Diels-Alder reaction (fig. 4)[10]. Owing to the presence of a double bond in ring B, two diene fragments are generated, the charge remaining preferentially with the left part of the molecule (*m/z* = 108) rather than the right fragment (*m/z* = 218) which in addition is subject to further decomposition, e.g. *m/z* =203, 189, 133[10]. Other fragments, such as *m/z* = 173 have their counterpart (*m/z* = 257) in the mass spectrum of olean-12-ene.

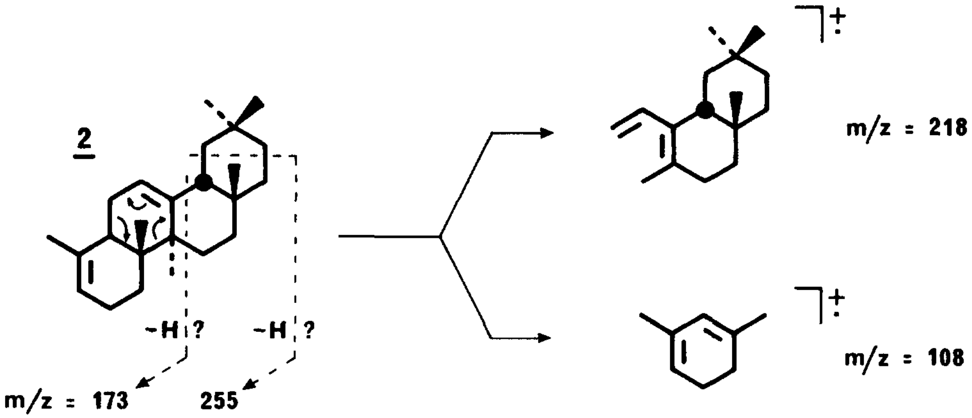


Figure 4 : Mass spectrometric fragmentations proposed for compound **2**.

^1H and ^{13}C NMR data are in complete agreement with the postulated structure and allowed assignment of most of the carbons and hydrogens. Analysis of the ^{13}C-NMR ^1H noise decoupled and DEPT spectra indicates that **2** contained six methyl, eight methylene, four methine groups and six quaternary carbons. The assignment of carbon signals was based on the results obtained from two-dimensional heteronuclear (^1H-^{13}C) chemical shift correlation experiments, after assignment of the ^1H signals. The ^1H-NMR spectrum (fig. 5a) shows the presence of two vinylic protons at δ_H 5.39 and 5.22 ppm, one methyl group on a double bond at δ_H 1.62 ppm and five methyls on quaternary carbons. The proton connectivity pattern was determined by application of two-dimensional homonuclear (^1H-^1H) correlation spectroscopy, using the COSY-90 sequence, and by ^1H homo-decoupling spectral measurements (schematised on figure 5b). For B and C rings, the assignment was started from the signal of the 25-CH_3. Connectivity between H-9 and 25-CH_3 was established by a ^1H selective decoupling experiment. Assignment of D and E ring protons required the unambiguous localisation of H-18, the signal of which overlaps with those of the two H-6 : this was carried out by using the results of heteronuclear (^1H-^{13}C) correlation experiments, H-18 being located from the last methine (C-18) not yet assigned. A long-range coupling ('W'-coupling) permitted to establish the connection between H-19β and H-21β. Two-dimensional nuclear Overhauser effect (NOESY) and n.O.e. difference experiments (fig. 5c) distinguished

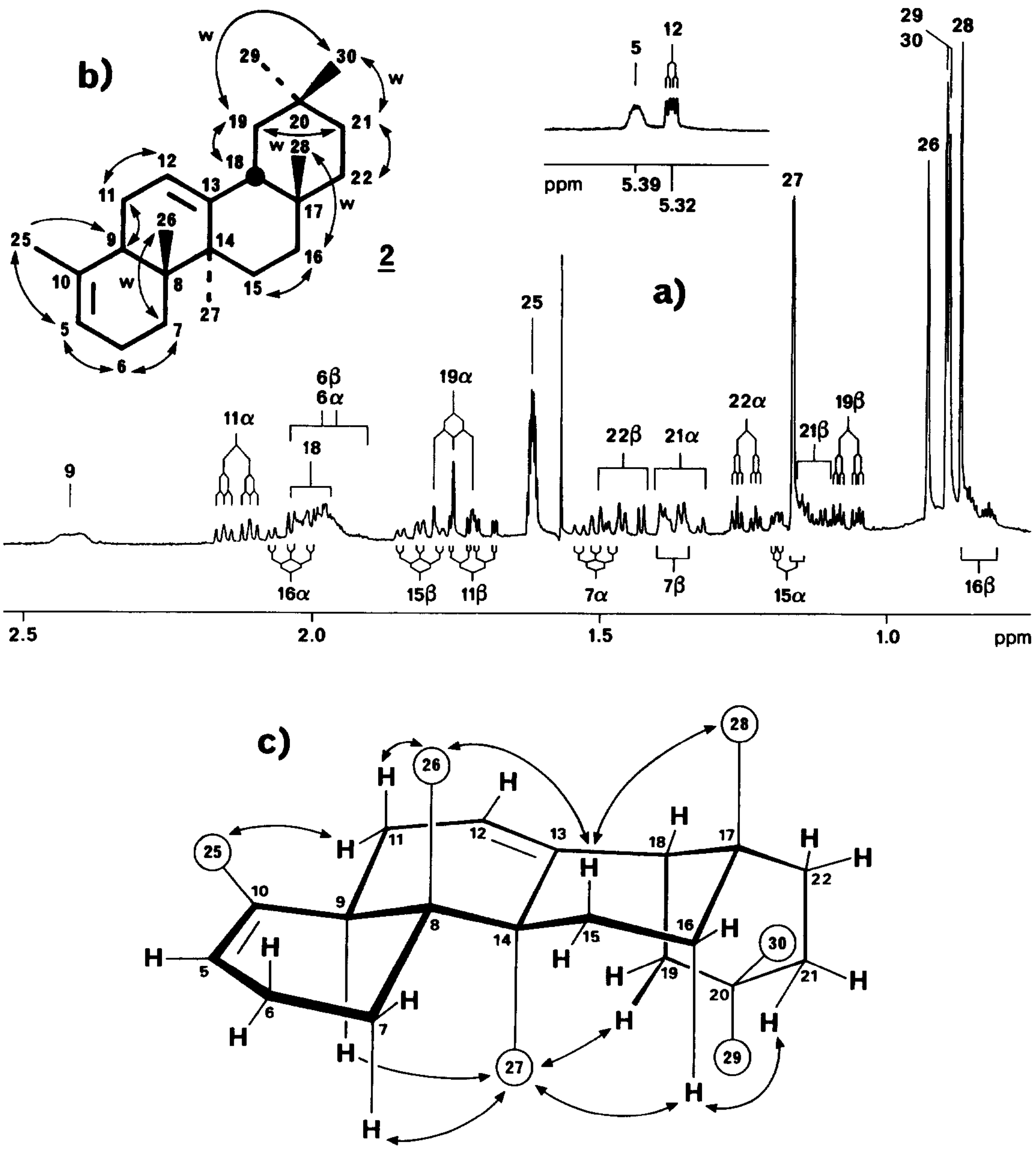


Figure 5 : ^{1}H-NMR spectrum and ^{1}H connectivity patterns observed for des-*A*-oleana-5(10),12-diene **2**.

a) ^{1}H-NMR spectrum (400 MHz, $CDCl_3$).

b) COSY and selective decoupling experiments (W = 'W'-coupling).

c) NOESY and n.O.e. difference experiments. Only the most essential connectivities are reported.

between the two pairs H-15 and H-16 and furnished further evidences strongly supporting the above assignments and giving valuable stéreochemical informations. Indeed, connectivities could be established between different parts of the molecule, e.g. n.O.e.'s between 25-CH_3/H-11β, H-11β/26-CH_3, 26-CH_3/H-15β, H-15β/28-CH_3, H-7α/27-CH_3, H-16α/27-CH_3, H-9/27-CH_3 (the latter by a n.O.e. difference experiment) and the cis-D/E ring junction was checked by n.O.e.'s of 27-CH_3 with H-19α and H-16α with H-21α.

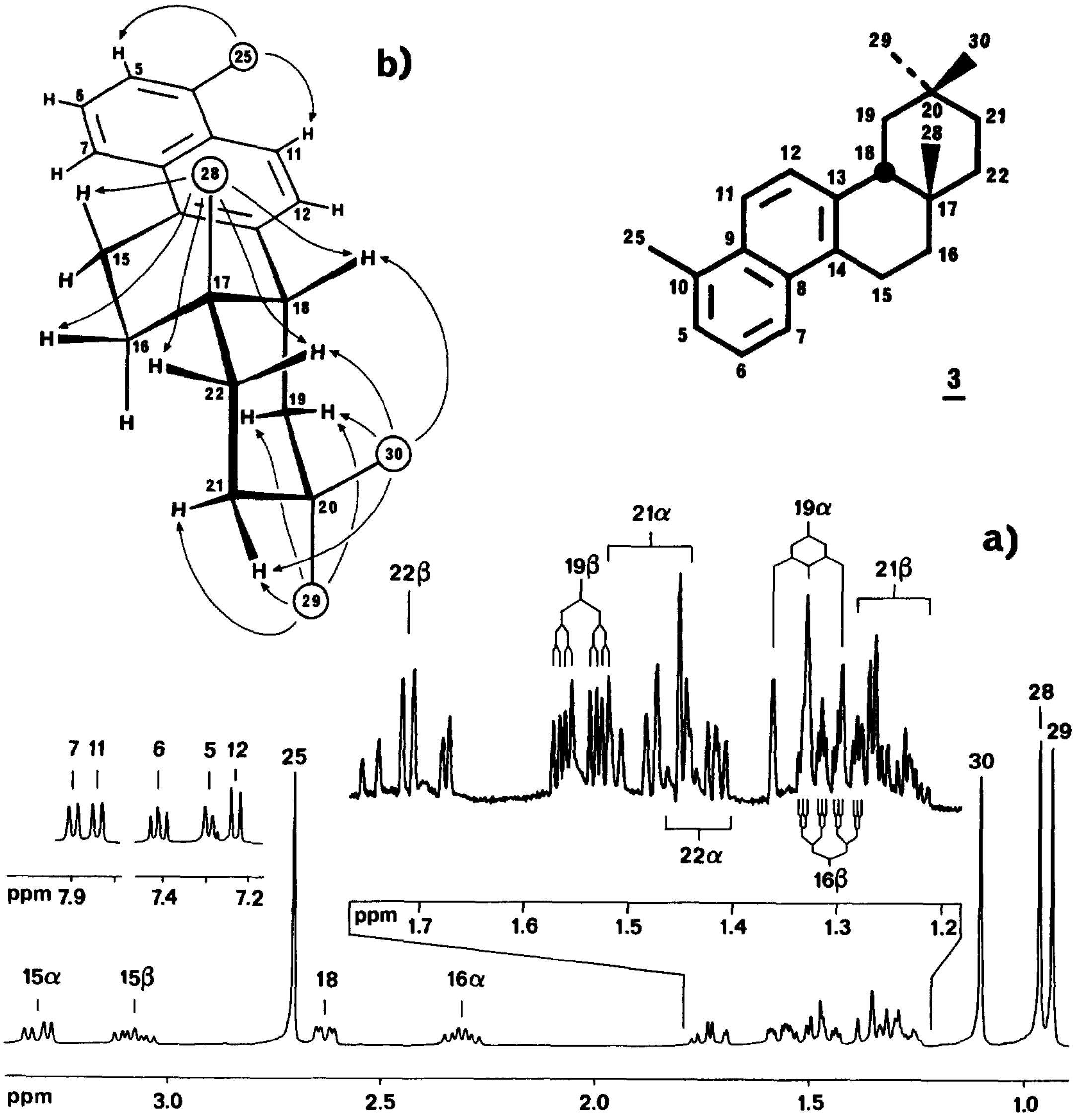


Figure 6 : a) ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of des-A-26,27-dinoroleana-5,7,9,11,13-pentaene 3.
b) ^{1}H connectivity pattern observed by n.O.e. difference experiments.

The structure of des-*A*-26,27-dinoroleana-5,7,9,11,13-pentaene **3** (fig. 6) was previously partially elucidated by comparison with a synthetic standard[3]. However, the stereochemistry of the D/E ring junction remained unknown for both the natural and synthetic compounds. We cleared this point by ^{1}H-NMR studies which also allowed assignment of all the protons as follows (fig. 6a). Protons at C-15 and C-16 were identified by successive selective decouplings : the resonances associated with H-15α and H-15β show downfield shifts to δ_H 3.29 and δ_H 3.06 ppm respectively, while the signal for H-16α is observed at δ_H 2.29 ppm. The signal of H-16β (δ_H 1.31 ppm), which partially overlaps with H-19α, appears as a kind of *ddt* and therefore exhibits an additional small coupling (*J ca* 1.6 Hz) which disappears upon irradiation of the last single benzylic proton (H-18, δ_H 2.61 ppm). This 'W'-coupling provides evidence for a cis-D/E ring fusion. The same argument has been used for the structural elucidation of the corresponding pentacyclic triaromatic component in the oleanane series[1b]. H-18 shows in addition two couplings with protons at C-19 (H-19α δ_H 1.55 ppm; H-19β δ_H 1.33 ppm). As in the case of the diene **2**, H-19β displays a 'W'-coupling (*J* 2.5 Hz) with H-21β. N.O.e. difference experiments involving the three non benzylic methyl groups definitively established the cis-stereochemistry of the D/E ring junction and permitted unambiguous assignment of protons at C-21 and C-22, as well as of the methyl protons. Observed connectivities are reported on figure 6b. Only the connectivity between H-21β and 30-CH_3 does not appear clearly. The assignment of the aromatic protons was based on a specific decoupling of H-6 (δ_H 7.38 ppm) which allowed location of the two vicinal protons H-7 and H-5. The latter was distinguished by a n.O.e. difference experiment irradiating the benzylic methyl group (25-CH_3, δ_H 2.69 ppm). This experiment also allowed distinction between H-11 and H-12.

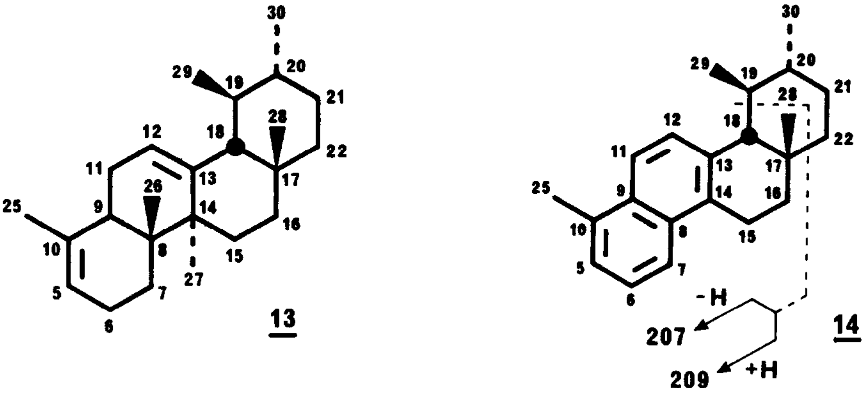
Ursane series

Three compounds related to the ursane series were isolated and identified by MS, ^{1}H-NMR and X-ray diffraction (fig. 7 and 8).

Des-*A*-ursa-5(10),12-diene **13** is the counterpart in the ursane series of structure **2** in the oleanane series. These two series differing only by the position of the methyl groups 29 and 30 on ring E, some analogy between their spectral data may therefore be expected. Indeed, the mass spectrum of **13** exhibits the same major peaks as for **2** with changes only in their relative intensity (see experimental part). Consequently, the same structural considerations are valid for both compounds. The ^{1}H-NMR spectrum of **13** shows also a striking analogy with the one of **2**. Resonances of all the protons belonging to rings B, C, and D (except H-7β which overlaps in both cases with protons of ring E) are similar in their form (multiplicity) and show only slight differences in their chemical shift (see experimental part). Two important differences should, however, be noted. First, all the protons bound to ring E seem to overlap in the region of 1.2 - 1.5 ppm and could therefore not be attributed. Second, two methyl groups (δ_H 0.93 and 0.82 ppm) appear as doublets (*J* 6 Hz), each of them coupling with a vicinal proton. It was not possible, of course, to precisely assign these two methyl groups on the basis of these data alone. The proposed structure seems nevertheless very likely, especially as the oxygenated precursors and other related hydrocarbons such as **14** and **15** are present in the investigated sediment. Indeed, occurrence of the two aromatic hydrocarbons **14** and **15** in recent or ancient sediments has been proposed before on the basis of data obtained by GC-MS analysis[9]. But a precise identification required more structural informations and in particular to elucidate the stereochemistry of the D/E ring junction for **14** and the position and the configuration of the two methyls on ring E for both compounds.

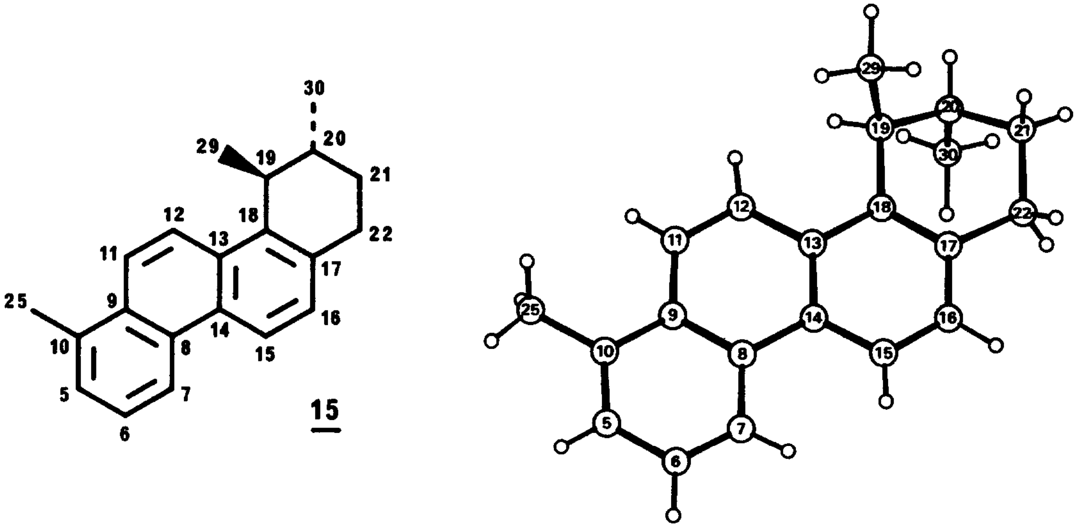
The mass spectrum of Des-*A*-26,27-dinorursa-5,7,9,11,13-pentaene **14** exhibits the same major fragments as **3** in the oleanane series, thus suggesting closely related compounds (see experimental part). However, fragmentations leading to ions *m/z* = 207 (base peak for **14**) and *m/z* = 209 appear to be strongly enhanced in the case of **14**. This is consistent with a substituent at C-19, which would probably favour the benzylic cleavage of the C-18 - C-19 bond (fig. 7). As in the case of the NMR study of compound **3**, protons at C-15 and C-16 were identified by successive decouplings of H-15α (δ_H 3.33 ppm), H-15β (δ_H 3.11 ppm) and H-16α (δ_H 2.43 ppm). As before, the signal of H-16β (δ_H 1.37 ppm) appears like a *ddt* with a 'W'-coupling (*ca* 1.5 Hz) which disappears upon irradiation of H-18 (2.07 ppm). This is again the first proof in favour of a *cis*-D/E ring junction. H-18 shows apparently only one other coupling (10 Hz) with a proton at C-19 (δ_H 1.16 ppm), the signal of which collapses into a triplet (*J ca* 10, 10 Hz) by decoupling of the methyl doublet at 0.88 ppm. This proves that this methyl (29-CH_3) is positioned at C-19 and suggests the presence of only one proton at C-20. The proton associated with the second methyl doublet (δ_H 0.95 ppm) appears as a *ddd* (δ_H 1.23 ppm, *J ca* 10, 10, 4 Hz) upon irradiation of the latter. Biosynthetic considerations and the fact that no or very small (< 1.5 Hz) coupling between H-19 and a possible second proton at C-20 would appear unlikely in this case led us to locate the second methyl group doublet (30-CH_3) at C-20. Value of couplings (10 Hz) of H-19 with H-18 and H-20 are consistent with a relative quasi *trans*-diaxial disposition for these protons, implying that 29-CH_3 and 30-CH_3 are in a equatorial position, respectively β and α refering to E ring. *Cis*-stereochemistry of the D/E ring fusion was further confirmed by a n.O.e. difference experiment irradiating the non-benzylic methyl group singlet (28-CH_3, δ_H 0.79 ppm) which shows connectivity with H-18.

The structure of des-*A*-26,27,28-trinorursa-5,7,9,11,13,15,17-heptaene **15** was established by X-ray diffraction on a monocrystal (fig. 8). As for most of the above described compounds, the absolute configuration of **15** is indicated by analogy with that attributed to the natural triterpene series. The quasi *trans*-diaxial disposition of the two vicinal methyl groups should be noted. This conformation also exists preferentially in solution as indicated by ^{1}H-NMR data. The benzylic proton H-19 appears as a broad *dq* (δ_H 3.42 ppm) which changes upon irradiation of the methyl group doublet at 1.42 ppm (29-CH_3). Resonance of H-20 (δ_H 2.10 ppm) was located by decoupling of H-19 and of the methyl group doublet 30-CH_3 (δ_H 1.07 ppm). The small coupling constant (*ca* 2 Hz) between H-19 and H-20 suggests that these protons are in an equatorial position : the respective methyls should therefore be in a quasi *trans*-diaxial conformation. The two C-22 benzylic protons appear at 2.92 (H-22β) and 3.02 (H-22α) ppm as *ddd* showing second-order features apparent as intensity distorsion, but their signal is otherwise almost symmetrical. The large coupling (*ca* 11 Hz) between H-22α and H-21β (2.18 ppm) is consistent with a *J*(ax,ax). H-21α is located at 1.64 ppm. N.O.e. difference experiment involving the 29-CH_3 shows a strong effect with H-21β which also confirms the preferential *trans*-diaxial conformation in solution of the two methyl groups at C-19 and C-20. Assignment of aromatic protons was based on n.O.e.'s between 29-CH_3/H-12, 25-CH_3/H-5, 25-CH_3/H-11, a selective decoupling of H-6 and the fact that the signal of H-15 should appear more deshielded compared with H-16.



13 **14**

Figure 7 : Structure and carbon numbering of hydrocarbons 13 and 14 of the ursane series.



15

Figure 8 :Carbon numbering and three-dimensional structural drawing of 15 obtained from X-ray crystallography. Atoms of ring E are more or less deviated from the mean plane of the three rings B, C and D (mean e.s.d. : 0.004 Å) : C-19: 0.045, C-20: -0.490, C-21: 0.189, C-22: -0.052, C-29: 1.477, C-30: -2.001 Å.

CONCLUSION

Eight tetracyclic hydrocarbons (saturated, olefinic, aromatic) related to three major higher plant triterpene series (lupane, oleanane, ursane) have been identified by X-ray diffraction, NMR and MS studies in a recent anoxic sediment from the Rhine valley.

These conclusive identifications confirm the general occurrence of loss of ring A and of subsequent aromatisations affecting higher plant triterpenes at an early stage of diagenesis in recent sediments. In particular

Δ5(10) olefinic compounds or their isomers, which retain almost the full structural information of the original triterpenes, appear to be key-intermediates leading in further stages either to saturated or to aromatic compounds. The predominance of polyaromatic components of the oleanane and ursane series is probably due to the presence of an additional double bond in the skeleton which seems to favour the aromatisation pathway. This predominance is in agreement with results obtained from incubation of labelled compounds. The availability of these pure compounds obtained by synthesis and/or isolation from the sediment was essential for the interpretation of our results from incubation experiments of radiolabelled triterpenes of the lupane and oleanane series. The latter have indeed clearly demonstrated that these degradation reactions are mediated by micro-organisms.

EXPERIMENTAL

General

Physical measurements : NMR spectra were recorded either on WP 200 SY or AM 400 Bruker instruments. Chemical shift values are internally referenced to the residual protons in the deuterated solvents. Digital resolution was generally set to 0.25 Hz per point. *J* values result from first-order interpretations and are given with a minimal precision of ± 0.5 Hz. Unless otherwise indicated, NMR spectra were obtained in $CDCl_3$ ($CHCl_3$ δ_H 7.27 ppm) solution at concentration of *ca* 4 to 15 x 10^{-3} M. Low-resolution electron impact mass spectra (LREIMS) were taken either on LKB 9000 S or Kratos MS 80 GC-MS systems. High-resolution mass measurements (HREIMS) were obtained on a Thomson CSF THN 208 mass spectrometer. Infrared spectra (IR) were taken on a Perkin-Elmer 177 spectrophotometer. Optical rotation measurements were performed on a Perkin-Elmer 241 MC polarimeter. Melting points (mp) were measured with a Reichert microscope and are uncorrected. Elemental analyses were done at the Service Central de Microanalyse du C.N.R.S. (division de Strasbourg). X-ray diffraction data were obtained by using a Philips PW 1100 diffractometer.

Chromatographic techniques : Column chromatography (CC) was carried out on Merck 40-63 µm silica gel 60 using the indicated solvent. Analytical and preparative thin-layer chromatography (TLC) was performed on precoated plates (0.25, 0.5 or 1 mm) of Merck silica gel 60 F_{254}. Silica gel-$AgNO_3$ plates (0.25 mm) were prepared by immersion of the precoated plates in a solution of $AgNO_3$ (10%) in ethanol-water (3:1) for 4 min. After drying at room temperature (48 h), they were reactivated for 1 h at 120°C. Gas chromatographic (GC) analyses were carried out on a Carlo Erba Fractovap 4160 chromatograph with on-column injector and flame ionisation detector and fitted with WCOT glass columns (*ca* 30 m x 0.25-0.3 mm, OV-73 or SE-54 phases obtained from Alltech Associates). Typical operating conditions were hydrogen carrier gas (*ca* 2-3 ml/min), temperature programmed from 50 to 100°C at 20°C/min and from 100 to 300°C at 3°C/min, detector heater temperature at 300°C. High performance liquid chromatography (HPLC) was made on a Waters equipment (Model 6000A or 590EF pumps, U6K injector, R401 differential refractometer). Columns and typical flow rates were Merck Hibar 250 x 4 mm RT LiChrosorb RP-18 5 µm, 1-2 ml/min; Merck Hibar 250 x 7 mm RT LiChrosorb RP-18 7 µm, 4-5 ml/min; Du Pont 250 x 9.4 mm Zorbax ODS 8 µm, 5 ml/min; Du Pont Golden series 80 x 6.2 mm Zorbax ODS 3 µm, 2 ml/min. Solvents used were methanol or a mixture methanol-water (up to 15%).

Isolation of tetracyclic des-*A*-triterpenoid hydrocarbons from the investigated sediment.

Extraction of the sediment . The fluid mud collected in a pond near Huttenheim (Alsace, France) was roughly filtered (*ca* 2 mm-mesh) in order to remove the most voluminous debris and then centrifugated. The wet sediment recovered (8.5 kg of mud still containing 80-82% water) was extracted using a procedure similar to that of Bligh and Dyer[11] A mixture of mud (250 g), methanol (500 ml) and chloroform (250 ml) was stirred for 1 h at 45-50°C. After centrifugation and transfer of the monophasic solution to a separatory funnel, chloroform (250 ml) was added (but no water which would cause emulsions). After blending and decantation allowing to obtain a biphasic system, the chloroform layer was recovered, washed with brine (1 l) and concentrated. The upper methanol-water layer was again extracted with chloroform (200 ml). The residue of centrifugation was then extracted with a mixture of chloroform-methanol (2:1, 900 ml) for 1 h at 45-50°C. After centrifugation, the liquid phase was washed with brine (1 l) and the chloroform layer concentrated. All the chloroform extracts (*ca* 40-50 g) were combined. For analytical GC-MS purpose, a small quantity of mud was freeze-dried. The powdery sediment obtained (95 g) was extracted twice with a mixture of chloroform-methanol (2:1, 600 ml) by stirring for 1 h, the first time at room temperature and then at 45-50°C.

Separation of the total extract into neutral and acidic fractions was carried out on silica gel impregnated with potassium hydroxide[12]. The neutral fraction was then fractionated on CC (silica gel, gradient of acetone in dichloromethane). The less-polar first-eluting fraction (dichloromethane) was again chromatographed on column (silica gel, gradient of diethylether in hexane). The head fraction (eluted with hexane) contained saturated and unsaturated (non-aromatic) hydrocarbons. The following fractions (eluted with up to 5% diethylether in hexane) consisted of aromatic hydrocarbons. Saturated and unsaturated hydrocarbons were separated on a silica gel-$AgNO_3$ (10%) column (hexane) which also allowed a first separation of unsaturated compounds among themselves (gradient of diethylether in hexane). Most of these olefinic products were further purified on TLC (silica gel/hexane, 0.25 mm). Finally pure hydrocarbons were obtained by fine separation using reverse-phase HPLC [Merck LiChrosorb RP-18 5 or 7 µm/methanol or methanol-water (up to 15%)]. Aromatic hydrocarbons fractions were also subjected to prep. TLC (silica gel/hexane or hexane-dichloromethane (up to 8%), 0.25 or 0.5 mm) and then to reverse-phase HPLC allowing pure compounds to be isolated. All compounds isolated had a purity of at least 99% as determined by GC analysis. About 15 mg of **12**, 15 mg of **2**, 1 mg of **13**, 4 mg of **3**, 2 mg of **14**, 15 mg of **15** were obtained. Four additional pure hydrocarbons were also isolated but their structure has not yet been completely elucidated.

Physical data of the isolated compounds.

Des-*A*-lup-9-ene (12) : ^1H-NMR and MS data are the same as those recorded for the synthetic compound within experimental error (see below). Mp : 117.5°C (from methanol-dichloromethane). $[\alpha]_D$: -107° (*c* 0.586, $CHCl_3$). X-ray crystallography : $C_{24}H_{40}$, M = 328.56. Crystals obtained by recrystallisation from a mixture of methanol and dichloromethane, prismatic 0.3 x 0.3 x 0.5 mm; orthorhombic system, space group $P2_12_12_1$, Z = 4. Cell parameters : a = 22.492(15), b = 11.268(9), c = 8.085(7) Å, V = 2049.06 Å^3, d_c = 1.06 gcm^{-3}, $\lambda(CuK\alpha)$ = 1.5418 Å. From the 2178 unique reflections collected by the θ - 2θ scan technique up to θ = 68°, 1848 were considered as observed [$I > 3\sigma(I)$]. Structure solved by direct methods[13] and refined by full-matrix least-squares minimizing $\Sigma w(\Delta F)^{2}$(ref 14). Hydrogen atoms were introduced in their theorical position (C-H = 1.08 Å) and assigned the equivalent isotropic thermal factor of the bonded C atom. Final conventional R was 0.0616, the weighting scheme being $w = 1/\sigma^2(F_0) + 0.000063F_0^2$. Lists of fractional atomic coordinates, bond distances and angles, anisotropic thermal factors, observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre, U.K., as supplementary material.

Des-*A*-oleana-5(10),12-diene (2) : NMR (^1H · 400 MHz, ^{13}C : 100.6 MHz) . all NMR experiments were performed using a sample of **2** (*ca* 10 mg, 0.031 mM) in $CDCl_3$ (*ca* 0.4 ml) in a 5-mm o.d. tube sealed under vacuum after purging with argon.

H	δ_H ppm	*J* Hz	H	δ_H ppm	*J* Hz	C	δ_C ppm	C	δ_C ppm
	$CHCl_3$ 7 27						$CDCl_3$ · 77 0		
5	5.39		18	2.01		5	121.1	17	38.0^c
6α	1.95-1.99		19α	1.76	13.5, 13.5	6	22.9	18	48.7
6β	1.95-1.99		19β	1.07	13.5, 4.5, 2.8	7	27.7	19	46.3
7α	1.51	12.0, 12.0, 6.0	21α	1.36		8	31.1^c	20	41.2^c
7β	1.37		21β	1.13		9	38.2	21	34.8
9	2.43		22α	1.25	13.0, 3.5, 3.5	10	136.0^b	22	37.1
11α	2.13	17.5, 6.0, 4.5	22β	1.46		11	27.1	25	21.0
11β	1.72	17.5, 12.5, 2.5	25	1.62		12	122.6	26	14.6
12	5.32	4.5, 2.5	26	0 93		13	145.6^b	27	24.6
15α	1.18	13.7, 4.2, 3.0	27	1 16	0.6	14	33.0^c	28	28.6
15β	1.81	13.7, 13.7, 4.2	28	0.87		15	26.4	29	23.7^d
16α	2.04	13.7, 13.7, 4.2	29	0.90^a		16	27.2	30	33.4^d
16β	0.84		30	0.89^a					

a-d · signals may be interchanged

GC-LREIMS (70 eV), *m/z* (rel. int.) : 326(M^+,24%), 311(25), 255(12), 243(2), 231(5), 229(7), 218(10), 215(3), 205(10), 204(16), 203(13), 189(10), 173(13), 161(5), 159(4), 147(9), 145(5), 133(12), 121(16), 119(16), 108(100), 95(70).

Des-*A*-26,27-dinoroleana-5,7,9,11,13-pentaene (3) : ^{1}H-NMR (400 MHz, $CDCl_3$) :

H	δ_H ppm CHCl3 : 7.27	*J* Hz	H	δ_H ppm	*J* Hz	H	δ_H ppm	*J* Hz
5	7.27	7.0	16α	2.29	13.4, 12.0, 6.9	22α	1.43	
6	7.39	7.0, 8.5	16β	1.31	13.4, 7.3, 1.6, 1.2	22β	1.71	
7	7.87	8.5	18	2.61	12.8, 4.5, 1.6	25	2.69	
11	7.81	8.7	19α	1.33	13.8, 12.8	28	0.94	
12	7.21	8.7	19β	1.55	13.8, 4.5, 2.5	29	0.91	
15α	3.29	17.7, 6.9, 1.2	21α	1.48		30	1.08	
15β	3.06	17.7, 12.0, 7.3	21β	1.25				

GC-LREIMS (70 eV), *m/z* (rel. int.) : 292(M^+, 100%), 277(5), 264(2), 263(2), 249(<1), 235(2), 221(7), 208(9), 207(16), 206(6), 205(6), 195(12), 193(16), 192(12), 191(9), 189(6), 181(20), 179(10), 178(15), 168(22), 165(13), 155(13), 153(8).

Des-*A*-ursa-5(10),12-diene (13) : ^{1}H-NMR (400 MHz, $CDCl_3$) : attribution by analogy with **2**

H	δ_H ppm CHCl3 7.27	*J* Hz	H	δ_H ppm	*J* Hz	H	δ_H ppm	*J* Hz
5	5.39		15α	1.18	13.5, 4.3, 2.0	22α	1.2-1.5	
6α	1.95-1.99		15β	1.87	13.5, 13.5, 4.5	22β	1.2-1.5	
6β	1.95-1.99		16α	2.04	13.5, 13.5, 4.3	25	1.62	
7α	1.49	12.6, 12.6, 6.1	16β	0.89		26	0.94^b	
7β	1.2-1.5		18	1.2-1.5		27	1.10^b	
9	2.38		19α	1.2-1.5		28	0.84^b	
11α	2.18	17.7, 5.5, 5.0	19β	1.2-1.5		29	0.93^c	6.0^a
11β	1.73	17.7, 12.5, 2.3	21α	1.2-1.5		30	0.82^c	6.0
12	5.25	5.0, 2.3	21β	1.2-1.5				

a : measured in a mixture of $CDCl_3$-C_6D_6 (1:2)
b, c : signals may be interchanged

GC-LREIMS (70 eV), *m/z* (rel. int.) : 326(M^+, 34%), 311(37), 255(17), 243(8), 231(5), 229(7), 218(11), 215(7), 205(5), 204(5), 203(8), 189(7), 173(16), 161(7), 159(6), 147(14), 145(9), 135(15), 133(21), 121(22), 119(28), 108(94), 95(100).

Des-*A*-26,27-dinorursa-5,7,9,11,13-pentaene (14) : ^{1}H-NMR (400 MHz, $CDCl_3$) :

H	δ_H ppm CDCl3 · 7.27	*J* Hz	H	δ_H ppm	*J* Hz	H	δ_H ppm	*J* Hz
5	7.27	6.9	16α	2.43	13.6, 9.0, 8.5	22α	1.41-1.64	
6	7.38	8.6, 6.9	16β	1.37	13.6, 9.0, 1.5, 1.5	22β	1.41-1.64	
7	7.75^a	8.6, 0.8	18	2.07	10.0, 1.5	25	2.70	
11	7.88^a	8.6	19	1.16		28	0.79	
12	7.21^a	8.6	20	1.23		29	0.88	6.2
15α	3.33	18.0, 8.5, 1.5	21α	1.41-1.64		30	0.95	6.1
15β	3.11	18.0, 9.0, 9.0	21β	1.41-1.64				

a : signals may be interchanged

GC-LREIMS (70 eV), *m/z* (rel. int.) : 292(M^+, 80%), 277(1), 235(2), 222(5), 221(7), 209(42), 208(37), 207(100), 206(8), 205(5), 195(11), 193(18), 192(16), 191(10), 189(5), 181(10), 179(14), 178(15), 168(22), 165(13), 155(7), 153(5).

Des-*A*-26,27,28-dinorursa-5,7,9,11,13,15,17-heptaene (15) : ^{1}H-NMR (400 MHz, $CDCl_3$) :

H	δ_H ppm CDCl3 - 7.27	*J* Hz	H	δ_H ppm	*J* Hz	H	δ_H ppm	*J* Hz
5	7.41	7.1	16	7.37	8.6	22β	2.92	*ca* 17.4, 6.5, 3.1
6	7.52	8.3, 7.1	19	3.42	3 x 6.8, 2.0	25	2.77	
7	8.57	8.3	20	2.10		29	1.42	7.1
11	7.99	9.5	21α	1.64	*ca* 13.5, 6.3, 3.5, 3.1	30	1.07	7.0
12	8.08	9.5	21β	2.18	*ca* 13.5, 10.8, 6.5, 3.8			
15	8.50	8.6	22α	3.02	*ca* 17.4, 10.8, 6.3			

GC-LREIMS (70 eV), *m/z* (rel int.) : 274(M$^+$, 79%), 259(100), 245(17), 232(5), 231(8), 230(14), 229(22), 228(13), 227(7), 226(6), 218(7), 217(15), 216(10), 215(21), 205(6), 203(5), 202(13), 189(6). X-ray crystallography : $C_{21}H_{22}$, M = 274.41. Crystals obtained by recrystallisation from a mixture of methanol and dichloromethane belong to the orthorhombic system, space group $P2_12_12_1$, Z = 4. Cell parameters : a = 8.258(4), b = 8.885(4), c = 21.243(6) Å, V = 1558.65 Å^3, d_c = 1.17 gcm^{-3}, λ(MoKα) = 0.7107 Å, μ = 0.33 cm^{-1} (absorption ignored). From the 1981 unique reflections collected by the θ - 2θ scan technique up to θ = 27°, 1417 were considered as observed having $I \geq 2.5\ \sigma(I)$ [σ(I) derived from counting statistics] and kept in the refinement calculations. The structure was solved by direct methods[13] and refined by full-matrix least-squares minimizing $\Sigma w(\Delta F)^{2(\mathrm{ref}\ 14)}$. All the hydrogen atoms were located on difference Fourier maps and refined with an isotropic thermal factor equivalent to that the carbon atom. Final conventional R was 0.0458, final weighting scheme : $w = 1/\sigma^2(F_0) + 0.0026F_0^2$, final residual electron density : between - 0.23 and 0.16 e Å^{-3}. Lists of the fractional atomic coordinates, bond distances and angles, anisotropic thermal parameters for the C atoms, observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre, U.K., as supplementary material.

Preparation of reference compounds 7, 11 and 12 in the lupane series.

(10βH)-Des-*A*-lupane (7) and Des-*A*-lupane (8) : Ketone **6** (100 mg, 0.291 mM) was heated for 5 h at reflux under argon in a mixture of diethylene glycol (20 ml), 1-butanol (15 ml) and hydrazine hydrate (15 ml, 309 mM). Excess of hydrazine, 1-butanol and water were removed by distillation, potassium hydroxyde (3 g) was added after cooling and the solution was heated at 200 °C for 5 h. The reaction mixture was allowed to cool to room temperature, diluted with water and extracted with ether (3 X). The combined organic extracts were washed with water and brine, dried ($MgSO_4$) and concentrated under reduced pressure. Purification by chromatography on a short column (silica gel/hexane) afforded hydrocarbons (90 mg of a white solid, 94%) which in GC-MS analysis were a mixture of **7** (*ca* 95%) and **8** (*ca* 5%). These two alkanes were separated by reverse phase HPLC (Du Pont Zorbax ODS/MeOH).

7 : Alkane of shorter retention time in GC (OV-73, SE-54) and HPLC (Du Pont Zorbax ODS/MeOH). ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 0.98 (3H, *s*), 0.94 (3H, *s*), 0.85 (3H, *d*, *J* = 6.9 Hz, H-29 or H-30), 0.80(3H, *d*, *J* = 6.2, H-25), 0.77 (3H, *s*), 0.77 (3H, *d*, *J* = 6.7 Hz, H-29 or H-30). GC-LREIMS (70 eV), *m/z* (rel. int.) : 330(M$^+$, 35%), 315(13), 287(27), 219(5), 217(5), 206(26), 193(13), 192(33), 191(31), 177(24), 163(61), 149(61), 137(29), 136(19), 135(15), 123(100), 109(82), 95(73), 81(70), 69(52), 67(37), 55(57). HREIMS (70 eV) M$^+$, *m/e* : 330.3273; $C_{24}H_{42}$ requires : 330.3286. Mp : 94-95 °C (from methanol-dichloromethane). Anal. Found : C, 86.95; H, 13.03. $C_{24}H_{42}$ requires : C, 87.19; H, 12.81%.

8 : ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 1.04 (3H, *s*), 0.94 (3H, *s*), 0.93 (3H, *d*, *J ca* 6-7.5 Hz, H-25), 0.85 (3H, *d*, *J* = 6.9 Hz, H-29 or H-30), 0.77 (3H, *d*, *J* = 6.7 Hz, H-29 or H-30), 0.76 (3H, *s*). GC-LREIMS (70 eV), *m/z* (rel. int.) : 330(M$^+$, 23%), 315(8), 287(16), 219(3), 217(2), 206(25), 193(8), 192(14), 191(22), 177(12), 163(54), 149(30), 137(23), 136(20), 135(11), 123(100), 109(77), 95(57), 81(60), 69(48), 67(37), 55(51).

(10βH)-Des-*A*-lupan-5α-ol (9) and **(10βH)-Des-*A*-lupan-5β-ol (10)** : To lithium aluminium hydride (30 mg, 0.79 mM) in anhydrous ether (10 ml) at room temperature under argon was added the ketone **6** (186 mg, 0.541 mM) in dry ether (10 ml). The mixture was stirred for 2 h at reflux and left at room temperature overnight. Excess of lithium aluminium hydride was destroyed with ice, the products were diluted with ether, washed with water and dried ($MgSO_4$). Chromatography of the products on prep. TLC (silica gel/CH_2Cl_2, 1 mm) afforded the two alcohols **9** (20 mg of a colorless oil, 11%) and **10** (143 mg of a white solid, 76%).

9 : TLC R_f : 0 4 (silica gel/CH_2Cl_2, 0.25 mm). ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 3.75-3.69 (1H, *m*, H-5), 1.00 (3H, *s*), 0.99 (3H, *d*, *J* = 0.4 Hz), 0.92 (3H, *d*, *J* = 6.6 Hz, H-25), 0.85 (3H, *d*, *J* = 6.8 Hz, H-29 or H-30), 0.78 (3H, *s*), 0.77 (3H, *d*, *J* = 6.7 Hz, H-29 or H-30). GC-LREIMS (70 eV), *m/z* (rel. int.) : 346(M^+, 27%), 331(5), 328(13), 313(7), 303(10), 285(11), 206(26), 193(11), 192(19), 191(26), 177(16), 163(51), 149(47), 136(41), 125(66), 123(87), 122(100), 121(43). HREIMS (70 eV) M^+, *m/e* : 346.3226; $C_{24}H_{42}O$ requires : 346.3235. IR ($CHCl_3$) : 3620, 3360-3540 cm^{-1}.

10 : TLC R_f : 0.2 (silica gel/CH_2Cl_2, 0.25 mm). ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 3.13-2.95 (1H, *m*, H-5), 1.02 (3H, *s*), 0.96 (3H, *d*, *J* = 5.7 Hz, H-25), 0.92 (3H, br *s*), 0.85 (3H, *d*, *J* = 6.8 Hz, H-29 or H-30), 0.77 (3H, br *s*), 0.77 (3H, *d*, *J* = 6.7 Hz, H-29 or H-30). GC-LREIMS (70 eV), *m/z* (rel. int.) : 346(M^+, 25%), 331(5), 328(18), 313(7), 303(16), 285(12), 246(8), 220(7), 219(9), 206(18), 193(18), 192(35), 191(29), 177(21), 163(48), 149(56), 136(40), 125(34), 123(100), 122(93), 121(47). HREIMS (70 eV) M^+, *m/e* : 346.3226; $C_{24}H_{42}O$ requires : 346.3235. IR ($CHCl_3$) : 3620, 3360-3540 cm^{-1}. Mp : 229-230 °C (from methanol-dichloromethane), lit.[8] mp : 225-229 °C. Anal. Found : C, 83.20; H, 12.33. *Calcd for* $C_{24}H_{42}O$: *C, 83.17; H,* 12.21%.

Des-*A*-lup-5(10)-ene (11) and **Des-*A*-lup-9-ene (12)** : A stirred solution of alcohol **10** (73 mg, 0.211 mM) in dry pyridine (2 ml) under argon was treated with phosphorus oxychloride (40 µl, 0.43 mM) at room temperature for 6 h. The reaction mixture was then poured into a cold saturated $NaHCO_3$ aqueous solution and vigorously stirred for 10 min. The ether extracts were washed with brine, dried ($MgSO_4$) and concentrated. Separation on CC [silica gel-$AgNO_3$ (10%)/hexane] afforded the alkenes **11** (23 mg of a white solid, 33%) and **12** (15 mg of a white solid, 22%). The reaction was incomplete.

11 : TLC R_f : 0.5 (silica gel-$AgNO_3$/hexane, 0.25 mm). ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 5.33 (1H, *m*, H-5), 2.29 (1H, *m*, H-9), 1.61 (3H, *m*, H-25), 0.97 (3H, *s*), 0.94 (3H, br *s*), 0.86 (3H, *d*, *J* = 6.8 Hz, H-29 or H-30), 0.80 (3H, *s*), 0.79 (3H, *d*, *J ca* 6.5 Hz, H-29 or H-30). GC-LREIMS (70 eV), *m/z* (rel. int.) : 328 (M^+, 30%), 313(11), 299(5), 285(7), 273(33), 260(3), 259(3), 232(7), 231(9), 217(6), 205(25), 191(15), 189(9), 177(11), 175(9), 163(17), 161(8), *149(32), 147(9), 136(100), 123(48), 122(40), 121(40), 109(40), 107(60), 95(97), 93(44), 91(25), 81(52).* HREIMS (70 eV) M^+, *m/e* : 328.3127; $C_{24}H_{40}$ requires : 328.3130. Mp : 174 °C (from methanol-dichloromethane). Anal. Found : C, 87.60; H, 12.14. $C_{24}H_{40}$ requires : C, 87.73; H, 12.27%.

12 : TLC R_f : 0.8 (silica gel-$AgNO_3$/hexane, 0.25 mm). ^{1}H-NMR (200 MHz, $CDCl_3$) : δ 2.53 (1H, *ddd*, *J* = 14.4, 5.2, 2.0 Hz), 1.61 (3H, br *s*, H-25), 1.20 (3H, *s*), 0.86 (3H, *d*, *J ca* 6.5-7 Hz, H-29 or H-30), 0.84 (3H, *s*), 0.80 (3H, *s*), 0.78 (3H, *d*, *J ca* 6.5-7 Hz, H-29 or H-30). GC-LREIMS (70 eV), *m/z* (rel. int.) : 328 (M^+, 18%), 313(4), 285(3), 231(1), 205(30), 191(10), 189(5), 177(7), 175(3), 163(8), 161(7), 149(25), 147(7), 136(100), 123(37), 122(20), 121(23), 109(22), 107(31), 95(30), 93(22), 91(14), 81(33). HREIMS (70 eV) M^+, *m/e* : 328.3127; $C_{24}H_{40}$ requires : 328.3130. Mp : 117-117.5 °C (from methanol-dichloromethane). $[\alpha]_D$: -109° (*c* 0.436, $CHCl_3$). Anal . Found : C, 87.84; H, 12.26. $C_{24}H_{40}$ requires : C, 87.73; H, 12.27%.

Acknowledgments ; we thank E. Krempp and R. Graff for technical assistance in NMR; P. Wehrung, R. Hueber and G. Teller for the mass spectral measurements.

REFERENCES

1. a) W. CARRUTHERS and D.A.M. WATKINS, *J. Chem. Soc.*, 724-729 (1964). b) A.L. CHAFFEE and C.J.R. FOOKES, *Org. Geochem.* **12**, 261-271 (1988). c) I.R. HILLS, E.V. WHITEHEAD, D.E. ANDERS, J.J. CUMMINS and W.E. ROBINSON, *J. Chem. Soc., Chem. Commun.*, 752-754 (1966). d) V. JAROLIM, K. HEJNO, F. HEMMERT and F. SORM, *Collect. Czech. Chem. Commun.* **30**, 873-879 (1965). e) R.E. LAFLAMME and R.A. HITES, *Geochim. Cosmochim. Acta* **43**, 1687-1691 (1979). f) J. RULLKÖTTER, D. LEYTHAEUSER and D. WENDISH, *Geochim. Cosmochim. Acta* **46**, 2501-2509 (1982). g) A. SAPTORAHARDJO, J.M. TRENDEL and P. ALBRECHT, *in* : *Le sondage Misedor*, Editions Technip, Paris, pp. 307-316 (1987). h) M. STREIBL and V. HEROUT, *in : Organic Geochemistry - Methods and Results* (Ed. by G. Eglinton and M.T.J. Murphy), Springer, Berlin, pp. 401-424 (1969). i) Y.L. TAN and M. HEIT, *Geochim. Cosmochim. Acta* **45**, 2267-2279 (1981). j) S.G. WAKEHAM, C. SCHAFFNER and W. GIGER, *Geochim. Cosmochim. Acta* **44**, 415-429 (1980).
2. C. SPYCKERELLE, A. GREINER, P. ALBRECHT and G. OURISSON, *J. Chem. Res. (S)*, 330-331 ; *(M)*, 3746-3777 (1977).
3. C. SPYCKERELLE, A. GREINER, P. ALBRECHT and G. OURISSON, *J. Chem. Res. (S)*, 332-333 ; *(M)*, 3801-3828 (1977).
4. P. ALBRECHT, M. DASTILLUNG, B. CORBET and J. RIOLO, *in : Géochimie organique des sédiments marins. D'Orgon à Misedor.* Ed. C.N.R.S., Paris, pp. 407-420 (1983).
5. B. CORBET, P. ALBRECHT and G. OURISSON, *J. Am. Chem. Soc.* **102**, 1171-1173 (1980).
6. G. OURISSON, P. ALBRECHT and M. ROHMER, *Trends Biochem. Sci.* **7**, 236-239 (1982).
7. M. ROHMER, M. DASTILLUNG and G. OURISSON, *Naturwissenschaften* **67**, 456-458 (1980).
8. G.V. BADDELEY, T.G. HALSALL and E.R.H. JONES, *J. Chem. Soc.*, 1173-1181 (1964).
9. F.R. AQUINO NETO, J.M. TRENDEL and P. ALBRECHT, *Tetrahedron* **42**, 5621-5626 (1986).
10. H. BUDZIKIEWICZ, J.M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688-3699 (1963).
11. E.G. BLIGH and W.J. DYER, *Can. J. Biochem. Physiol.* **37**, 911-917 (1959).
12. R.D. McCARTHY and A. DUTHIE, *J. Lipid Res.* **3**, 117-119 (1962).
13. C. RICHE, program DEVIN, *7th European Crystallographic Meeting, Abstracts*, Jerusalem, p. 25 (1982).
14. G.M. SHELDRICK, SHELX76, program for crystal structure determination, University of Cambridge, U.K. (1976).