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4α-METHYLSTEROLS IN LACUSTRINE ENVIRONMENTS

N. Robinson

Instituto de Química — UFRJ. Centro de Tecnologia. Cidade Universitária, Ilha do Fundão. 21910 Rio de Janeiro, Brasil
Permanent address: University of Bristol. Organic Geochemistry Unit. Cantocks Close BS8 - 1TS. Bristol, England

ABSTRACT

Evidence concerning the source of 4α-methylsterols in lacustrine environments is reviewed. Strong proof for a dinoflagellate origin is provided by the distributions of 4α-methylsterols in natural populations of freshwater dinoflagellates and the underlying bottom sediments from two lakes, the 4α-methylsterol content of a culture of the freshwater dinoflagellate *Woloszynskia coronata,* and other published data. 4α-Methylsterols have also been shown to occur esterified with fatty acids in three freshwater species of dinoflagellates, and in this form may provide another input to sediments. A major dinoflagellate contribution to the Eocene lacustrine Messel oil shale can be recognised from the distribution and abundance of 4α-methylsterols. The geological fate of these compounds is discussed.

RESUMO

Este trabalho revê as evidências relativas à fonte de 4α-metilesteróis em ambientes lacustres. As distribuições de 4α-metilesteróis em populações naturais de dinoflagelados de água doce e de sedimentos de fundo de dois lagos, assim como o teor de 4α-metilesteróis de uma cultura de dinoflagelados de água doce *Woloszynskia coronata,* e outros dados publicados, constituem uma forte prova de que esses compostos se originam de dinoflagelados. Também é mostrado que os 4α-metilessteróis ocorrem esterificados com ácidos graxos em três espécies de dinoflagelados de água doce e, sob esta forma, podem, também, ser incorporados aos sedimentos. A distribuição e abundância de 4α-metilesteróis, presentes no folhelho betuminoso de Messel, do Eoceno, evidenciam uma grande contribuição a partir dos dinoflagelados. A evolução geológica desses compostos é discutida neste trabalho.

INTRODUCTION

4-Methylsterols have a widespread, but not ubiquitous occurrence in lacustrine sediments (Mattern *et al.,* 1970; Cranwell, 1982, 1984; Mermoud *et al.,* 1982; Robinson *et al.,* 1984, 1986, 1987a, b) and are always found as the 4α isomers. They are rare in terrestrial organisms, occurring at trace levels in some higher plants and mammalian tissue; for example, they have been reported to occur in the skin of citrus fruits (Mazur *et al.,* 1958; Williams *et al.,* 1967). Prior to 1976, the only 4-methylsterols detected in algae were $\Delta^{8(9)}$ unsaturated 4α-methylsterols, present as minor constituents in *Porphyridium cruentum* (unicellular red alga; Beastall *et al.,* 1974; Minale & Sodano, 1976) and *Euglena gracilis* (green alga; Beastall *et al.,* 1974).

The presence of 4-methylsterols as significant lipid components was first recognised in the methylotrophic bacterium *Methylococcus capsulatus,* which contains 4α-methyl-5α(H)-cholest-8(14) en-3β-ol (IIIa) (Bird *et al.,* 1971; Bouvier *et al.,* 1976). This led to the suggestion that bacteria might be the source of the sedimentary 4α-methylsterols (Dastillung *et al.,* 1980). There are, however, several indications to the contrary (see Brassell & Eglinton, 1983); namely, sedimentary 4α-methylsterols consist of a wide variety of structural types (C_{28}-C_{31}) dominantly ring saturated, IIIa has never been found as a major sedimentary component, 4α-methylsterols have been detected in sediment trap material (Wakeham *et al.,* 1980; Gagosian *et al.,* 1983) suggesting a pelagic algal source.

In 1976, 4α, 23, 24R-trimethyl-5α(H)-cholestan-3β-ol (dinosterol, Ic) was identified in the dinoflagellate *Gonyaulax tamarensis* (Shimizu *et al.,* 1976). Dinoflagellates are the only organisms reported to produce this structurally-characteristic sterol; hence, it was proposed to be a marker for contributions from dinoflagellates to sediments of the Black Sea (Boon *et al.,* 1979). Since 1976, studies of other marine species have resulted in the identification of a large number of 4α-methylsterols (reviewed by Withers, 1983). Many of these compounds have been found in marine sediments, supporting the idea of dinoflagellates as the major source (Boon *et al.,* 1979; Leeuw *et al.,* 1983; Brassell & Eglinton, 1983).

The evidence for dinoflagellates being similarly the major source of 4-methylsterols in lacustrine sediments is reviewed herein.

EXPERIMENTAL

Details of experimental methods have been published previously (e.g. Robinson *et al.,* 1984, 1987c), but, briefly, involve extraction of lipids with organic solvents, fractionation by column or thin layer chromatography (TLC), and analysis of sterols as their trimethylsilyl (TMS) derivatives by gas chromatography (GC) and GC-mass spectrometry (GC-MS), with cochromatography with standards when available.

RESULTS AND DISCUSSION

Sources of 4α-methylsterols in lake sediments

Firm evidence for the biological origin of 4α-methylsterols in the lacustrine environment was first obtained from a study of the lipid geochemistry of Priest Pot, a small hypereutrophic lake in the English Lake District (Robinson *et al.,* 1984). Analysis of the sterols of a natural population of the dinoflagellate *Peridinium lomnickii* collected from the waters of the lake and underlying bottom sediments, revealed a close similarity in distributions (Fig. 1). This constituted the first report of 4-methylstanols in a freshwater alga.

All of the sterols of *P. lomnickii* have also been identified in marine dinoflagellates and the 4α-methylsterols were composed of C_{28}-C_{30} ring saturated compounds and Δ^5-dinosterol (IIc), as characteristically found in marine species and marine and lacustrine sediments.

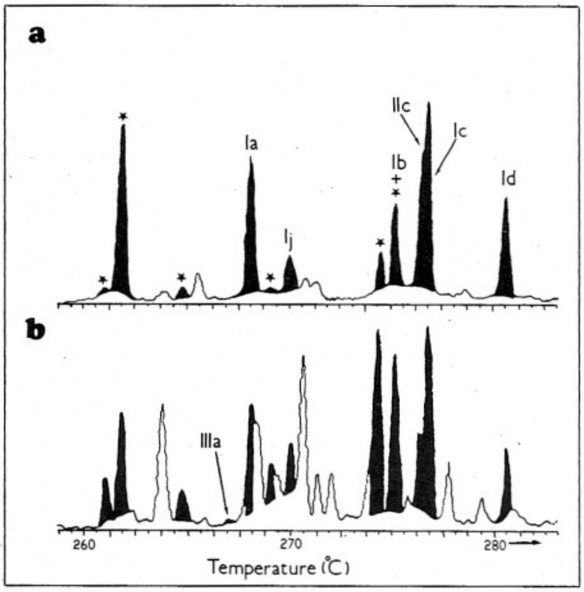

Figure 1 — Partial reconstituted ion chromatogram from GC-MS analysis of the alcohols (as TMS ethers) of (a) *P. lomnickii* (b) Priest Pot sediment (0-6 cm) free lipids. Shaded peaks represent sterols, Roman numerals refer to the structures of the 4α-methylsterols in the appendix and an asterisc indicates a desmethylsterol. Modified from Robinson *et al.* (1984).

Confirmation of a general dinoflagellate origin for 4α-methylsterols in lake sediments was provided by a study (Robinson *et al.*, 1986) of Lake Kinneret, Israel, in which productivity is dominated by the dinoflagellate *P. cinctum* (up to 99% phytoplankton biomass, 88% total biomass; Serruya, 1978). A natural population of *P. cinctum* from the lake was found to contain a range of C_{28}-C_{30} 4α-methylsterols, including the structurally-unusual peridinosterol (Ig) (Robinson *et al.*, 1986), previously only isolated from two marine binucleate dinoflagellate species (Withers, 1983). Comparison of the distribution of 4α-methylsterols in *P. cinctum* with that of the surface and 15 cm sediments (Fig. 2), demonstrates that all of the compounds present in *P. cinctum* are also present in the sediments with a similar distribution. Peridinosterol appears to be preferentially degraded, probably as a result of the lability of the $\Delta^{17(20)}$ unsaturation. The greater relative abundance of dinostanol (Id) in the sediments, and the small increase in abundance of this compound between the surface and 15 cm deep sediments, suggests that it may be formed by transformation of other sterols, for example, reduction of dinosterol or peridinosterol. Two additional C_{31} 4α-methylsterols (Ih and IIh) were present in the sediments; their origin is probably dinoflagellate, as biosynthesis of such cyclopropyl-containing sterols appears to be restricted to these organisms, particularly dinoflagellate zooxanthellae (Withers, 1983).

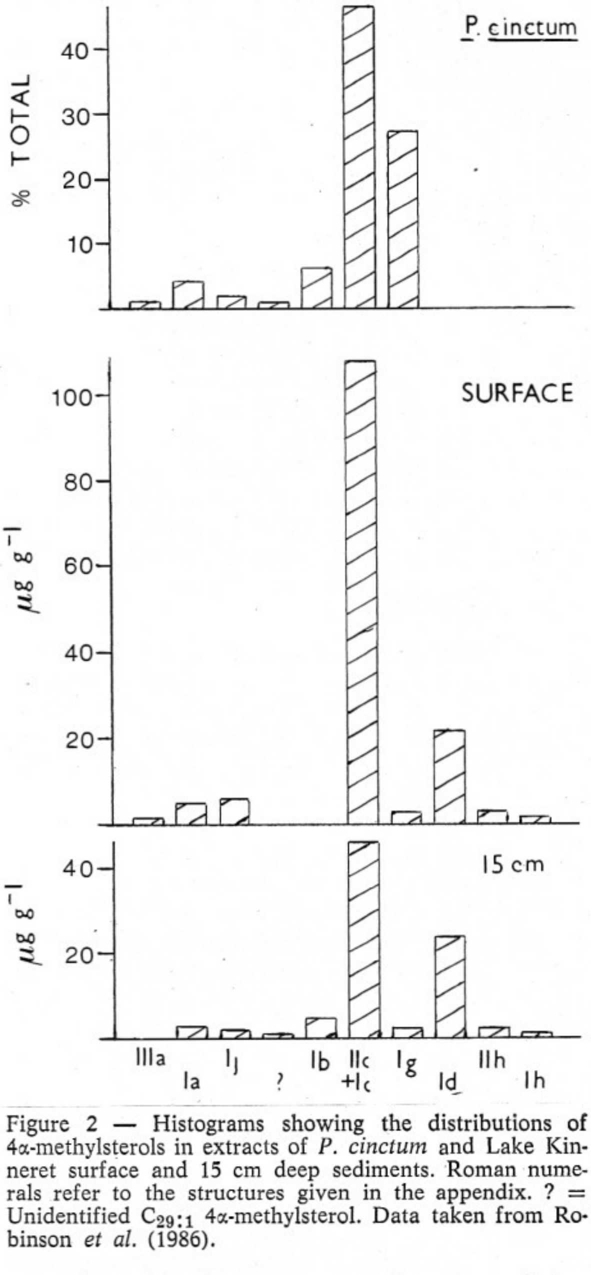

Figure 2 — Histograms showing the distributions of 4α-methylsterols in extracts of *P. cinctum* and Lake Kinneret surface and 15 cm deep sediments. Roman numerals refer to the structures given in the appendix. ? = Unidentified $C_{29:1}$ 4α-methylsterol. Data taken from Robinson *et al.* (1986).

The other known potential source of 4α-methylsterols is *M. capsulatus,* which produces C_{28} $\Delta^{8(14)}$ 4α-methylsterols (Bouvier *et al.*, 1976). A low concentration of 4α-methyl-5α(H)-cholest-8(14)-en-3β-ol (IIIa) was present in the sediments of Priest Pot, but not in *P. lomnickii* (Fig. 1). Hence, bacteria such as *M. capsulatus* could be the source of this sterol, although it may possibly originate from a different growth stage of *P. lomnickii* or from a different dinoflagellate species, since it also occurs in *P. cinctum.* The sediments of Coniston Water, an oligo-mesotrophic lake, contain low levels of IIIa as the only 4α-methylsterol (Robinson *et al.*, 1987a). In this case, a bacterial source is indicated by the absence of a suite of other 4α methylsterols expected from dinoflagellates and by the fact that the lake does not support a significant dinoflagellate population.

At this stage it is pertinent to consider the case of Lake Léman and Voua de la Motte, in which sedimentary 4α-methylstanols were attributed to a *de novo* bacterial biosynthesis, based on their absence in both potential external sources such as plankton and in the products of sterol incubation in the sediments (Mermoud *et al.*, 1982). At the time of this work, several of the compounds were unknown in organisms, however, all have since been isolated from dinoflagellates (e.g. Withers, 1983; Robinson *et al.*, 1987c). Dinoflagellate blooms may be aggregated both vertically and horizontally (George & Heaney, 1978) and may be short-lived. Hence, plankton sampling at three week intervals, as made during the study of sterols in Lake Léman, might miss a dinoflagellate bloom and its component 4α-methylsterols. In addition, the photosynthetic dinoflagellate *Gymnodinium arenicolus* has been reported to inhabit interstitial waters of Lake Léman (Dragesco, 1965) and, presumably, would not have been present in the plankton tow samples taken by Mermoud *et al.* (1982). Alternatively, the plankton population of Lake Léman may have changed in recent years, as 4α-methylsterols were not detected in the upper 3 cm of the sediments.

Cultures of three other species of freshwater dinoflagellates have been studied for their lipid content. In the case of *Ceratium hirundinella* (Cranwell, 1976), the TLC fraction corresponding in polarity to 4α-methylsterols was a minor component compared to desmethylsterols, and was not analysed. In another study (Robinson *et al.*, 1987c), *Woloszynskia coronata* was found to contain a simple distribution of sterols, consisting solely of 4α-methyl compounds (Ia, 40%; Ib, 19%; IIc, 38%; Ic, 3%), whereas *C. furcoides* only possessed desmethylsterols, although these included analogues of 4α-methylsterols characteristic of dinoflagellates.

Table 1 — Distribution of 4α-methylsteryl esters in three species of freshwater dinoflagellates.

Compound[1]	Abundance (% of total) P.l.	P.c.[2]	W.c.
4-Me C_{28}-14:0	5	3	4
4,24-diMe-14:0	4	6	1
4,23,24-triMe $\Delta^{5,22}$-14:0		21	7
4,23,24-triMe Δ^{22}-14:0	4	tr	
4,23,24-triMe-14:0	5		
4-Me C_{28}-16:0	13	3	27
4,24-diMe Δ^{22}-16:0	tr	tr	
4,24-diMe-16:0	9	6	4
4,23,24-triMe $\Delta^{5,22}$-16:0	2	15	19
4,23,24-triMe Δ^{22}-16:0	13	tr	
4,23,24-triMe-16:0	11		
4-Me C_{28}-18:1	5	tr	11
4,24-diMe-18:1		3	2
4,23,24-triMe $\Delta^{5,22}$-18:1		32	25
4,23,24-triMe Δ^{22}-18:1	4	tr	tr
4,23,24-triMe-18:1	5		
4-Me C_{28}-20:1	16		
Unidentified	4	12	

1. Shorthand notation in the form alkyl-acyl, e.g. 4,23, 24-triMe Δ^{22}-14:0 refers to 4,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol esterified to a satured *n*-C_{14} fatty acid. Identifications were made by comparison of mass spectra with those of standards or by spectral interpretation. tr = trace (1%)
2. Unidentified 4-methylsteryl esters with shorter acyl chains also present

Data taken from Robinson *et al.* (1987c)

4α-Methylsteryl esters

4α-Methylsterols esterified with fatty acids have been isolated from *P. lomnickii, P. cinctum* and *W. coronata* (Robinson *et al.*, 1987c) with distributions (Table 1) similar to the corresponding free sterols. Thus, 4α-methylsteryl esters represent a potential dinoflagellate input to lacustrine sediments. Indeed, in the marine environment, such compounds have been recognised in Black Sea sediments and were proposed to be sourced from dinoflagellates (Leeuw *et al.*, 1983). Hydrolysis within the sediments could then provide a post-depositional input of 4α-methylsterols, although the steryl ester linkage can survive for at least 50000 years in lacustrine sediments (Cranwell, 1986).

4α-Methylsterols in ancient lacustrine sediments

Ancient sediments that have experienced a sufficiently mild thermal history may still contain functionalised lipids. One example is the lacustrine Eocene Messel oil shale, which has as major lipid components a suite of 4α-methylsterols (e.g. Mattern *et al.*, 1970; Habermehl & Hundrieser, 1983; Robinson *et al.*, 1987b) that probably originated from dinoflagellates. The sterols of Messel shale are dominated by C_{28}-C_{31} 4α-methyl compounds, including dinosterol (Table 2). One unusual feature of the distribution is the presence of 4α-methyl, 24-ethyl-5α (H) cholestan-3β-ol (If) as the major compound rather than the more common dinostanol (Id). If has been isolated in low amounts only from two marine species of dinoflagellates (Withers, 1983). This compound, however, was reported to be a major compound in the sediments of Lake Léman (Mermoud *et al.*, 1982) and the 4α- and 4β-methyl, 24-ethylcholestanes, derived from such a sterol (see following section), were shown to be present in Toarcian sediments of the Paris Basin (Wolff *et al.*, 1986b). Considering that If coelutes with Id on nonpolar capillary columns (Robinson *et al.*, 1987b) it may be more common than previously thought, particularly in view of the relatively few dinoflagellate species studied for their sterol content. Alternatively, the fact that dinoflagellates are thought to have first colonised lacustrine environments during the earliest Cenozoic, based on the lack of Cretaceous or earlier non marine species in the fossil record (Tappan, 1980), suggests the possibility that there has been a change in the average sterol composition of lacustrine dinoflagellates since the Messel shale was deposited, as a result of evolution, i.e. species that inhabited the Messel palaeo-lake are now extinct or do not compete successfully with evolutionary newer species. If dinoflagellates did not colonise the lacustrine environment until the Cenozoic, then 4α-methylsteroids might be expected to be absent from lacustrine sediments of greater age; to the best of the author's knowledge, this has proved to be the case up to the present, with the exception of some weathered residues of submarine oil seeps in the Otway Basin, Australia, tentatively identified as originating from lacustrine source rocks of Late Jurassic to Late Cretaceous age (McKirdy & Morton, 1985). In view of the uncertainty over the age of the source rocks it is tempting to suggest a later age based on the presence of 4-methylsteranes in the oils, but it should be stressed that the dinoflagellate fossil record is not complete and "may be more biased than that of such other major phytoplankton groups as the diatoms or coccolithophorids" (Tappan, 1980).

Table 2 — Abundance of 4α-methylsterols in two samples of the lacustrine Eocene Messel oil shale.

Compound[1]	Abundance (ug.g−1) Shale 1	Shale 2
Ia	38	100
Ib	18	61
Ic[2]	22	81
Ie	9	30
If[2]	94	376
Ii[3]	8	25
Ii[3]	7	22

1. See appendix of structures
2. Identifications confirmed by cochromatography with an authentic standard
3. Tentatively identified by mass spectral interpretation

Data taken from Robinson *et al.* (1987b)

Geological fate of 4α-methylsterols

As a result of numerous studies, the low temperature diagenetic pathways followed by 4-desmethylsteroids are now reasonably well understood (reviewed by Mackenzie *et al.*, 1982; see also Brassell *et al.*, 1984). In brief, reduction and dehydration of sterols produces Δ^2 sterenes; isomerisation gives the more stable Δ^4 and Δ^5 counterparts in a 60:40 mix; acid catalysed backbone rearrangement can produce diasterenes, at first retaining the biologically inherited 20R stereochemistry, but subsequently isomerising to a 1:1 mixture of 20R and 20S diasterenes; finally, reduction of the sterenes and diasterenes yields steranes and diasteranes, respectively, which can undergo isomerisation at positions 14 (13 in diasteranes), 17, 20 and 24.

While the presence of 4-methylsteranes and 4-methyldiasteranes in ancient sediments and oils (Rubinstein & Albrecht, 1975; Ensminger *et al.*, 1978; Mackenzie *et al.*, 1982; Wolff *et al.*, 1986a, b) testifies to the analogous diagenetic pathways operating for sedimentary 4α-methylsterols, these have been much less well understood than in the case of the desmethylsteroids. In part, this was due to the surprisingly low occurrence of 4-methylsterenes found in sediments (Gagosian *et al.*, 1980; Mackenzie *et al.*, 1982). Recent work by Wolff *et al.* (1986a, b) involving laboratory simulations of the low temperature diagenetic reactions of 4-methylcholest-4-ene, has demonstrated that 4α-methylsteroids do indeed undergo analogous acid catalysed reactions analogous to the desmethylsteroids, except that the major product of the initial equilibrium mixture was 4-methylscholest-4-ene itself, with only minor amounts of 4α- and 4β-methylscholest-5-enes and two ring-A contracted compounds. Similar distributions of C_{28} and C_{30} steroidal hydrocarbons have been found in Messel shale (Wolff *et al.*, 1986a; Robinson *et al.*, 1987b). Furthermore, evidence of the similarity in pathways of 4-methylsteroids and desmethylsteroids, was provided by the identification of a 4-methylspirosterene as a minor product of the backbone rearrangement of 4-methylcholest-4-ene in the laboratory and its presence in a Toarcian black shale (Wolff *et al.*, 1986b), analogous to the desmethylspirosterenes identified previously in immature sediments (Peakman *et al.*, 1984). Wolff *et al.* (1986b) suggested that the generally low 4-methylsterene:desmethylsterene ratio in sediments might be due to a slower rate of dehydration for 4α-methylsterols because of unfavourable stereochemistry, compared with 5α-(H)-stan-3α-ols, 4α-methyl analogues of which have never been observed. This is consistent with the very much higher 4α methylstanol:desmethylsterol ratio than 4-methylsterene:desmethylsterene ratio in Messel shale (Robinson *et al.*, 1987b).

CONCLUSIONS

The absence of significant amounts of suites of 4α-methylsterols in organisms other than dinoflagellates, the high correlation of structures with the sedimentary compounds, the close similarity in distribution of the 4α-methylsterols of the natural population of dinoflagellates and the bottom sediments in two lakes, and the absence of significant concentrations in sediments of lakes that do not support a major dinoflagellate population, provide convincing evidence that dinoflagellates are the major source of 4α-methylsterols in the lacustrine environment. Input of 4α-methylsteryl esters from dinoflagellates is another potential source of such compounds. Upon burial, 4α-methylsterols follow diagenetic pathways analogous to those of the desmethylsterols, eventually forming 4α-methylsteranes and 4α-methyldiasteranes, present in mixtures of isomers tending towards the thermodynamically most stable forms with increasing depth. The presence of 4α-methylsteroids in a sediment or oil, may well be a better marker of input from dinoflagellates than the fossil record based on morphological studies of fossilised dinoflagellate cysts.

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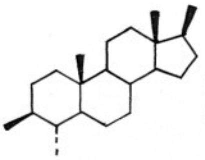
APPENDIX



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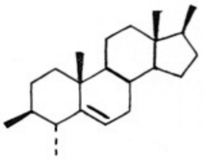

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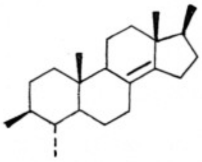

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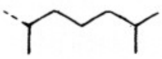

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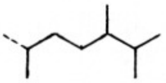

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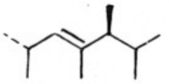

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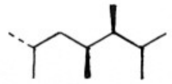

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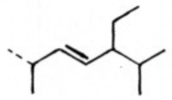

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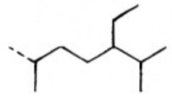

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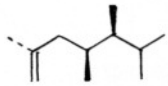

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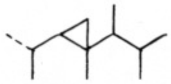

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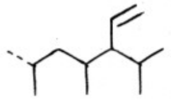

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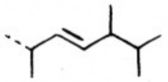

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