Phytochemistry, 1980, Vol. 19. pp. 791–793. Pergamon Press Ltd. Printed in England.

REGULARITY OF ISOPRENOID BIOSYNTHESIS IN THE ETHER LIPIDS OF ARCHAEBACTERIA

MARIO DE ROSA, AGATA GAMBACORTA and BARBARA NICOLAUS

Laboratory for the Chemistry of Molecules of Biological Interest, CNR, Via Toiano 2, Arco Felice, Naples, Italy

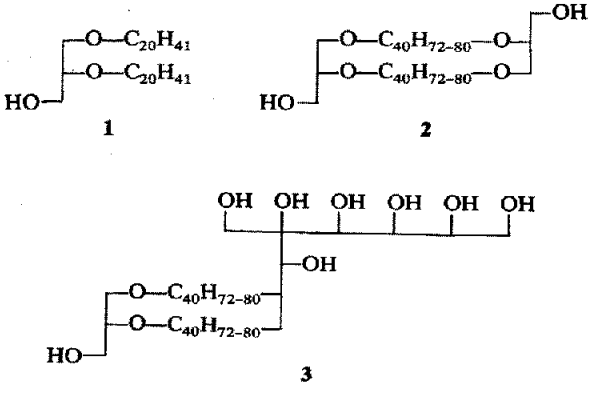
(*Received* 1 *August* 1979)

Key Word Index—*Caldariella acidophila*; archaebacteria; thermophilic bacteria; isoprenoid biosynthesis.

Abstract—The location of paired and unpaired ^{13}C atoms in the 16,16′-biphytanyl components of the lipids of *Caldariella acidiophila* following incorporation of acetate-[1,2-$^{13}C_2$] shows that the overall process of isoprenoid biosynthesis in this archaebacterial species follows a normal pattern and that the head-to-head linkage of the two tetraprenyl chains occurs stereoselectively.

The taxon of archaebacteria, which at present comprises methanogenic, halophilic, and thermoacidophilic species of considerable variety, has been segregated from the classically recognized bacteria on grounds which imply very prolonged evolutionary separation [1]. One characteristic common to all the archaebacteria is that their cell membrane is based upon ether lipids which, irrespective of complex lipid structures, are derived from the three structural types **1–3**.

In the glycerol diether (**1**) the hydrocarbon chains have the phytanyl (perhydrogeranylgeranyl) structure; this type of lipid occurs in the extreme halophiles [2] and in the methanogens [3]. The diglycerol tetraethers (**2**) also occur in the methanogens [3] but were first found in extreme thermoacidophiles of the *Caldariella* group [4–6] in which the glycerol calditol tetraethers (**3**) have also been characterized [6, 7]. These tetraethers all contain two C_{40} chains having the 16,16′-biphytanyl skeleton; the corresponding hydrocarbons have structures **4–8**, [5, 7]. However, in the methanogens, only type **4** has so far been reported [3] and in the lipids these chains are linked to the polyols at the 1,1′ termini. In the course of structural studies on the lipids of the *Caldariella* species of extreme thermoacidophiles the general isoprenoid nature of the C_{40} diols has been confirmed by incorporation of acetate-[^{14}C] and mevalonate-[^{14}C] and of acetate-[1-^{13}C] and acetate-[2-^{13}C] [7, 9]. The experiments also established that the individual isoprene units are formed by way of mevalonate and are labelled from acetate in the normal fashion, m_2c–mc (m = methyl, c = carboxyl).

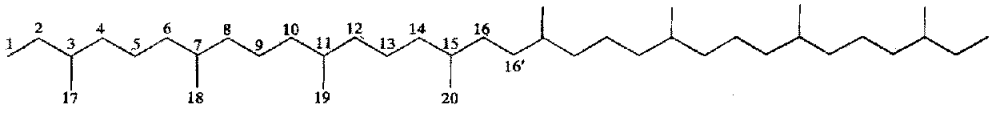


Since the archaebacteria plausibly represent the oldest examples of the evolution of the isoprenoid pathway, any further details have some general interest. The fully reduced C_{40} chains in **2** and **3** give no *a priori* indication of stereochemical constraints at the C_5 pyrosphosphate or prenyltransferase stages; equally there is no indication as to which two of the four possible methyl groups (in two presumed tetraprenyl intermediates, cf. (**1**)) are utilized in forming the unique 16,16′ head-to-head linkage.

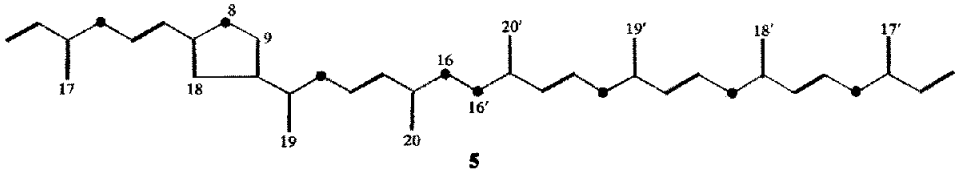
As the ^{13}C NMR spectra of the hydrocarbons **4–8** had been fully assigned in our structural investigations [7, 9], it was convenient to clarify these biogenetic problems by studying the incorporation of acetate-[1,2-$^{13}C_2$] into the lipids of *Caldariella acidophila* MT-4, growing at 85° and pH 3.5. It is well known that this label allows the carbons derived from C-2 of mevalonate to be distinguished from those from C-3′ by observation of the ^{13}C-^{13}C couplings in 'intact' acetate residues.

RESULTS

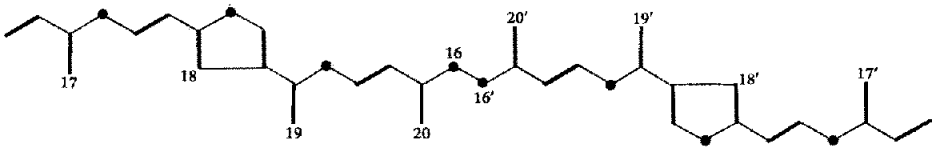
In preliminary work supplying a constant amount of acetate-[1,2-^{14}C], neither the % incorporation (1.4–1.6) nor the dilution of the label were affected by up to 40 μmol per l. of unlabelled acetate. The acetate-[1,2-$^{13}C_2$] (3.6 mmol, 90% ^{13}C) was added to a 90 l. batch of *C. acidophila* during the logarithmic growth phase; to minimize losses due to the volatility of acetic acid, normal aeration was reduced to 0.5 l. per min and the label added slowly through a peristaltic pump. At the end of logarithmic growth the cells were harvested (20 g lyophilized cells) and worked up as previously described [10], converting the total lipids (1.6 g) into the corresponding C_{40} hydrocarbons which were separated by preparative-scale GLC to give **4** (trace amount, insufficient for NMR), **5** (30 mg), **6** (390 mg), **7** (170 mg), and **8** (43 mg) (total recovery, 60%). The p.n.d. ^{13}C NMR spectra of labelled **5–8** were determined on 30–200 mg samples in 0.5 ml $CDCl_3$. In all



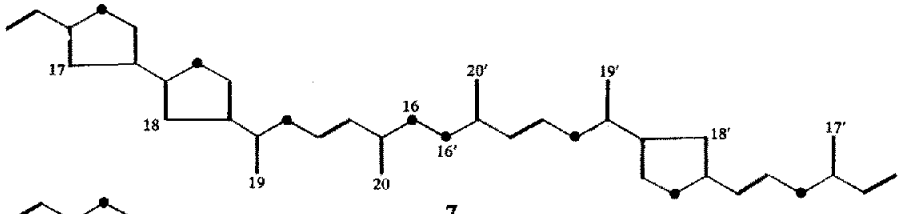
4



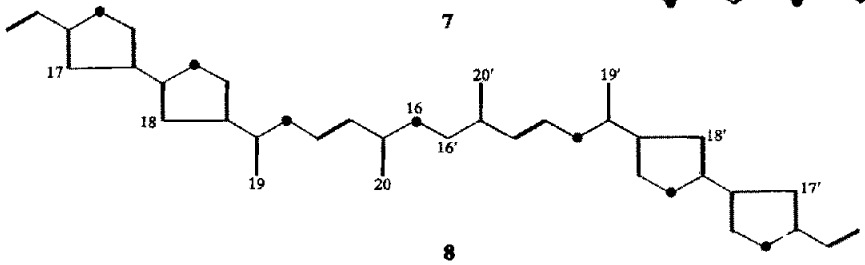
5



6



7



8

four spectra [7, 9] the signals from carbons 4,4′, 8,8′, 12,12′, 16 and 16′ all appeared as singlets of enhanced intensity; the remaining 32 carbons all gave rise to signals with satellite pairs due to ^{13}C-^{13}C couplings ($J = ca$ 35 Hz). The paired and unpaired carbon atoms were located as shown in the structures **5–8**.

The unpaired carbon atoms are those which derive from C-2 of mevalonate, and their 'in-chain' location in **5–8** shows that the assembly of the tetraprenyl components has followed the normal pattern, for example as in all-*trans*-geranylgeraniol. The relationship between the structures with CHMe and those with cyclopentane rings is confirmed, while the fact that the signals from the central carbons, 16 and 16′, are also unpaired shows that the head-to-head coupling reaction (whatever its mechanism) occurs with sufficient stereospecificity to conserve the separate identities of the original terminal methyls.

It therefore appears that the basic stereochemical constraints in isoprenoid biosynthesis are the same in 'archaebacteria' as in eubacteria and eukaryotes, a conclusion of some evolutionary significance.

Acknowledgements—We thank Dr. J. D. Bu'Lock for helpful discussion and E. Esposito, S. Sodano and R. Turco for technical assistance.

REFERENCES

1. Woese, C. R., Magrum, L. J. and Fox, G. E. (1978) *J. Mol. Evol.* **11,** 245.
2. Kates, M., Palameta, B., Joo, C. N., Kushner, D. J. and Gibbons, N. E. (1966) *Biochem. J.* **5,** 4092.
3. Tornabene, T. G. and Langworthy, A. T. (1979) *Science* **203,** 51.

4. De Rosa, M., Gambacorta, A., Millonig, G. and Bu'Lock, J. D. (1974) *Experientia* **30,** 866.
5. De Rosa, M., De Rosa, S., Gambacorta, A., Minale, L. and Bu'Lock, J. D. (1977) *Phytochemistry* **16,** 1961.
6. De Rosa, M., De Rosa, S., Gambacorta, A., and Bu'Lock, J. D. (1977) *J. Chem. Soc. Chem. Commun.* 514.
7. De Rosa, M., De Rosa, S., Gambacorta, A. and Bu'Lock, J. D. (1980) *Phytochemistry* **19,** 249.
8. De Rosa, M., Gambacorta, A., Minale, L. and Bu'Lock, J. D. (1974) *J. Chem. Soc. Chem. Commun.* 543.
9. De Rosa, M., De Rosa, S. and Gambacorta, A. (1977) *Phytochemistry* **16,** 1909.
10. De Rosa, M., Gambacorta, A. and Bu'Lock, J. D. (1976) *Phytochemistry* **15,** 143.