$$= -R \left\{ \frac{d \ln k_1'}{d\left(\frac{1}{T}\right)} + \frac{d \ln K}{d\left(\frac{1}{T}\right)} \right\} E_1' + \Delta H^\circ$$

where E_1' = activation energy for the redox decomposition of "oxyheme," and ΔH° = heat of formation of "oxyheme."

References

Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., and Shore, V. C. (1960), *Nature 185*, 422.

Kendrew, J. C., Watson, H. C., Strandberg, B. E., Dickerson, R. E., Phillips, D. C., and Shore, V. C. (1961), *Nature 190*, 666.

Lind, J. E., and Fuoss, R. M. (1961), *J. Phys. Chem. 65*, 999.

Paul, K.-G., Theorell, H., and Åkeson, Å. (1953), *Acta Chem. Scand. 7*, 1284.

Perutz, M. F., Rossmann, M. G., Cullis, A. F., Muirhead, H., Will, G., and North, A. C. T. (1960), *Nature 185*, 416.

Phillips, J. N. (1960), *Rev. Pure Appl. Chem. 10*, 35.

Shirley, E. L., and Blinn, F. V. (1954), A Method for the Determination of Dissolved Oxygen in Water, Schenectady, N.Y., Knolls Atomic Power Laboratory.

Sturtevant, J. M., and Spencer, T. (1959), *J. Am. Chem. Soc. 81*, 1874.

Wang, J. H., Nakahara, A., and Fleischer, E. B. (1958), *J. Am. Chem. Soc. 80*, 1109.

Wang, J. H. (1958), *J. Am. Chem. Soc. 80*, 3168.

Studies on the Mechanism of Biological Carbon Alkylation Reactions*

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ABSTRACT: Tuberculostearic acid (10-methylstearic acid) was isolated from *Mycobacterium smegmatis* grown in the presence of methionine-methyl-d_3. Mass spectrometry of the methyl ester of this fatty acid revealed the absence of any trideuterated species. A molecular peak corresponding to the dideuterated species, and less intense peaks corresponding to a monodeuterated species and a nonisotopic species, were observed.

An analogous experiment with ergosterol isolated from *Neurospora crassa* grown in the presence of methionine-methyl-d_3 gave essentially the same result. A molecular peak corresponding to the D_2 compound, but none corresponding to the D_3 compound, was found.

The classical studies of Keller *et al.* (1949) and duVigneaud *et al.* (1956) established that the methyl group of methionine could be transferred as a complete unit to various nitrogenous acceptors. By the use of methionine doubly labeled in the methyl group with both ^{14}C and deuterium, it was clearly established that deuterium atoms were not lost from the methyl group during transmethylation. Other investigations (Dewey *et al.*, 1954; Byerrum *et al.*, 1954; Sato *et al.*, 1957) have strengthened the idea that transfer of an intact methyl group is the normal course of biological transmethylation reactions.

In 1954, a new type of biological transalkylation[1] was postulated by Birch *et al.* (1954), and later demonstrated experimentally by Birch *et al.* (1957, 1958)—a reaction in which a methyl group of methionine is added to a presumably unsaturated carbon atom resulting in a methyl side chain. In more recent times several examples of this and slightly different carbon alkylation reactions have been discovered. These are summarized in Table I, with references to some of the known examples (see also, Mudd and Cantoni, 1964).

Since these carbon alkylation reactions are very

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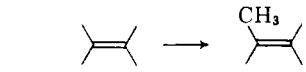
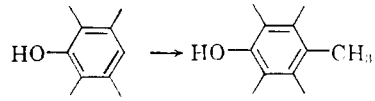
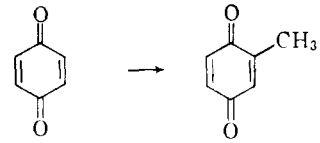
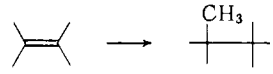
[1] In this paper the terms "alkylation" and "transalkylation" refer to any process which results in a methylated or alkylated product, regardless of the over-all mechanism of reaction. It is intended to include classical transmethylation reactions as well as transmethylenations of the type studied here and of the cyclopropane fatty acid synthetase type.

TABLE I: Carbon Alkylation Reactions from Methionine.

Reaction Type	Example	Reference
1	 RNA uridine → RNA thymine riboside	Fleissner and Borek (1962, 1963) Gold *et al.* (1963), Tropp *et al.* (1964)
	 mycophenolic acid	Birch *et al.* (1954, 1957, 1958) Jauréguiberry *et al.* (1964a)
	 coenzyme Q	Rudney and Parson (1963)
2	 oleic acid → tuberculostearic acid	Lennarz *et al.* (1962)
	? → ergosterol	Alexander and Schwenk (1957, 1958) Alexander *et al.* (1957, 1958) Parks (1958)
3	 vaccenic acid → lactobacillic acid	Liu and Hofmann (1962), Zalkin *et al.* (1963), Pohl *et al.* (1963)

different from the methylation of nitrogen, oxygen, and sulfur, it might be suspected that the mechanisms of these reactions would differ from the classical transmethylation reactions, and, indeed, that there might be different mechanisms for different carbon alkylation reactions. Some insight into this question can be gained from experiments which would show whether or not these reactions involve transfer of intact methyl groups.

In the case of a reaction of type 2, an attempt was made by Alexander and Schwenk (1958) to establish whether the methyl group was transferred from methionine as an intact unit. A yeast extract was incubated with a mixture of [methyl-^{14}C]methionine and [methyl-^{3}H]methionine of exactly known isotope content and isotope ratio. The ergosterol was isolated and its isotope ratio was determined. The ratio of tritium to carbon-14 in the ergosterol was approximately 90% of that in the methionine. Alexander and Schwenk (1958) concluded that this result was consistent with a transfer of all three protons on the methyl group, but they point out that an isotope effect in the breaking of carbon-hydrogen bonds was not considered.

However, since an isotope effect would be expected if carbon-hydrogen bonds were broken during the transalkylation reaction, it is evident that a retention of 90% of the tritium-carbon-14 ratio in the product could signify that one proton was lost, but that the remaining protons became enriched in tritium because the carbon-tritium bonds break much less readily than the carbon-hydrogen bonds. For example Pohl *et al.* (1963) have studied a reaction of type 3, in which obviously only two of the three protons of the methyl group can be transferred, and this was confirmed in an experiment with methionine-methyl-d_3. However, when the same sort of experiment was carried out with a mixture of [methyl-^{14}C]methionine and [methyl-^{3}H]methionine, the two protons of the product were so much enriched in tritium that the tritium-carbon-14 ratio of the product was 90% that of the methionine mixture.

In order to clarify the nature of reactions of type 2, we have undertaken an investigation of two reactions of this category, using methionine completely labeled with deuterium in the methyl group in order to eliminate ambiguities caused by an isotope effect. The reactions chosen were the formation of the 10-methyl group of tuberculostearic acid and the C-28 methyl group of ergosterol, since these groups originate from the methyl group of methionine (Alexander and Schwenk,

1957, 1958; Alexander *et al.*, 1958; Parks, 1958; Lennarz *et al.*, 1962). The results of the experiments described here show clearly that these reactions involve the transfer of the methyl carbon with only two of its protons.

Since the publication of a preliminary account of these experiments (Jauréguiberry *et al.*, 1964b), it has been demonstrated that in two cases of a reaction of type 1, the conversion of uridine to thymine riboside in soluble RNA and the formation of mycophenolic acid, a transfer of an intact methyl group to carbon takes place (Tropp *et al.*, 1964; Jauréguiberry *et al.*, 1964a).

Materials

Methionine-methyl-d_3 was prepared by the method of Melville *et al.* (1947), starting either with *S*-benzylhomocysteine (Cyclo Chemical Co., Los Angeles, Calif.) or with homocystine (Nutritional Biochemicals Corp., Cleveland, Ohio), and iodomethane-d_3 (Commissariat à l'Energie Atomique [C.E.A], Saclay). The twice-recrystallized product in either case gave one ninhydrin-positive spot corresponding to methionine in the paper chromatographic system of Schlenk and De Palma (1957). Mass spectrometry of the ethyl esters showed that the samples consisted solely of the trideuterated species.

Synthetic tuberculostearic acid, which was used for comparison with the deuterated sample, was the gift of Professor S. Hünig.

Methods

Growth of Organisms. Mycobacterium smegmatis was grown in a medium similar to that of Brodie and Gray (1956), but without Tween. Methionine was added at levels of 100 mg/liter for the L compound or 200 mg/liter for the DL compound. Growth was conducted in Roux bottles for 14 days at 37°, after which the cells were harvested by filtration.

Neurospora crassa, ATCC No. 10780,[2] was grown on the medium of Horowitz (1947) with either 20 mg/liter of L-methionine or 50 mg/liter of DL-methionine. Growth was conduced in 10-liter bottles which contained 6 liters of medium, with vigorous aeration, at 30°. The mycelium was harvested by filtration at the end of 4 days of growth.

Saponification. The wet cells were suspended in methanol (2 ml/g of wet cells) and solid KOH (1 g/g of wet cells) was added. The suspension was shaken until the KOH had dissolved and then the mixture was heated under reflux for 2–3 hours.

Isolation of Lipids. The basic hydrolysate of *M. smegmatis* was acidified and the lipids were extracted with ether. After evaporation of the ether, the crude lipids were extracted with boiling methanol, in which ordinary fatty acids are soluble, but the mycolic acids are insoluble. The methanol-soluble acids were esterified with diazomethane, and the esters were purified further by passing them over a silicic acid column, 2 × 4 cm, packed in benzene. The esters eluted with benzene were subjected to a gas-liquid chromatography. A column (0.95 × 244 cm) packed with 10% SE-30 (General Electric Co.) on Chromosorb (Johns-Manville Corp.) was operated at 200° under a helium pressure of 1–2 kg/cm². A Chromagas instrument, Model CG-1 (Profit, Paris), equipped with a stream-splitting device and a hydrogen-flame ionization detector, was used. The esters were trapped in glass spiral tubes as they emerged from the column. After two passes through the column the esters were generally at least 98% homogeneous.

The basic hydrolysate of *N. crassa* was extracted with ether for removal of the nonsaponifiable fraction. The crude nonsaponifiable fraction (100-200 mg) was applied to a column of silicic acid (Mallinckrodt 2847, sieved to 100–200 mesh), 2 × 4 cm, packed in benzene. After elution of pigments and hydrocarbons with benzene, the crude sterol fraction was eluted with benzene-ether (95:5). The crude ergosterol was recrystal-

TABLE II: Principal Peaks from the Mass Spectrum of Methyl Tuberculostearate.[a]

m/e	Identity
74 (r)	$CH_3O\overset{\overset{OH}{|}}{C}(\overset{2}{=}CH_2)$
87	$CH_3OC(=O)(CH_2)_2$—
143	$CH_3OC(=O)(CH_2)_6$—
167	Formed by loss of CH_3OH from *m/e* 199
171	$CH_3OC(=O)(CH_2)_8$—
199	$CH_3OC(=O)(CH_2)_8\overset{\overset{CH_3}{|}}{C}H$—
269 (M − 43) (r)	Formed by loss of $(\overset{2}{C}H_2—\overset{3}{C}H_2—\overset{4}{C}H_2 + H)$ from M
281 (M − 31)	Formed by loss of CH_3O— from M
283 (M − 29) (r)	Formed by loss of $(\overset{2}{C}H_2—\overset{3}{C}H_2 + H)$ from M
312 (M)	Molecular ion

[a] Indication of positive charge is omitted; (r) indicates formation through a rearrangement process. See Ryhage and Stenhagen (1963).

[2] This organism was isolated as a methionine auxotroph (Horowitz, 1947). The strain used in these experiments was apparently either a temperature-sensitive mutant or a revertant to wild type, for it showed good growth in the absence of methionine, especially when it was grown at 23°. At 30° there was very little growth in the absence of methionine, but it appears that some methionine was produced by the cells even at 30°.

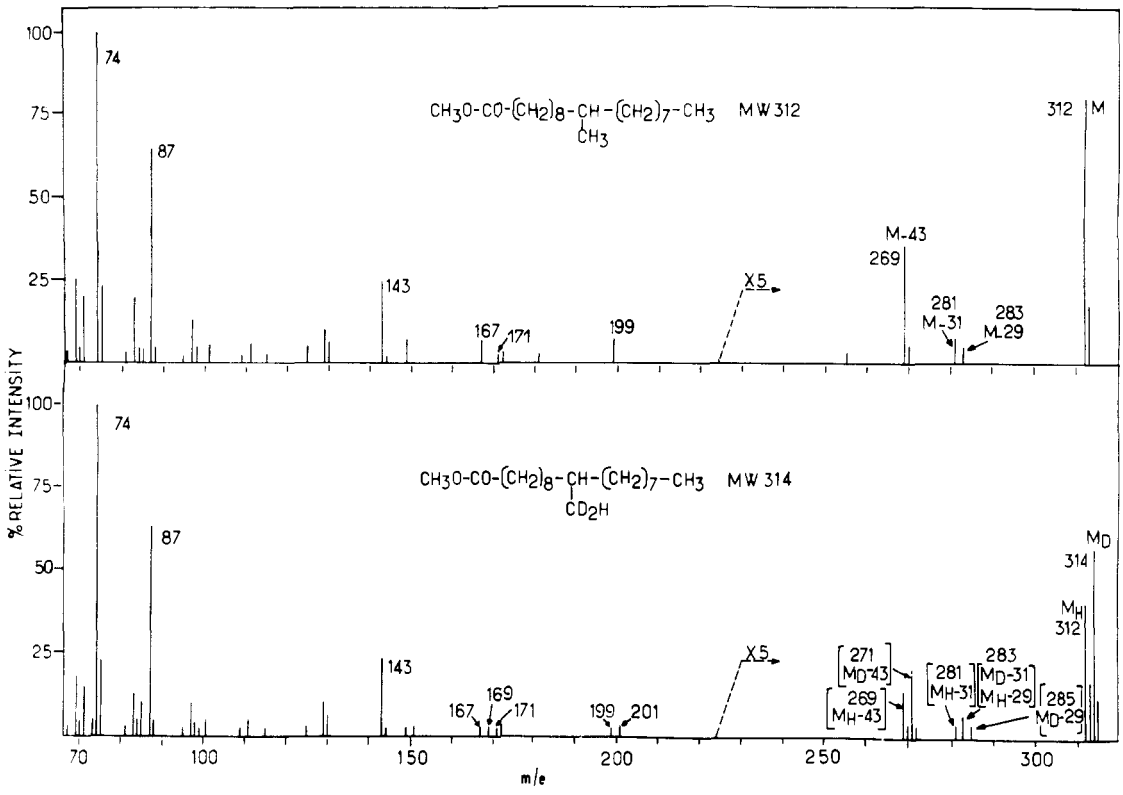


FIGURE 1: Mass spectra of methyl tuberculostearate. Upper spectrum (1a), synthetic compound. Lower spectrum (1b), compound isolated from cells grown on methionine-methyl-d_3.

lized from methanol 4–6 times to give a reasonably pure product as judged by melting point, ultraviolet spectrum, and gas-liquid chromatography.

Mass Spectrometry. The Atlas-Werke Model CH-4 mass spectrometer was used. The inlet and reservoir temperatures were 240°, and the ion source was maintained at 260°. The electron energy was 70 ev.

Results

Methyl Tuberculostearate. The methyl ester of synthetic tuberculostearic acid and the ester of the acid isolated from *M. smegmatis* were subjected to mass spectrometry. The spectra are shown in Figure 1. Extensive investigations of the mass spectra of fatty acid methyl esters (Ryhage and Stenhagen, 1963) and methyl tuberculostearate in particular (Ryhage and Stenhagen, 1960) allow the assignment of structure to virtually every significant peak in the mass spectrum of this latter compound. Table II summarizes the identity of ions found in the mass spectrum of methyl tuberculostearate (Figure 1a). Comparison of the mass spectra shows that many peaks in Figure 1b appear as "doublets" in the ratio of approximately 1:1.3, compared with Figure 1a, owing to the presence of a mixture of labeled and unlabeled methyl tuberculostearate. Ions of *m/e* 74, 87, and 143 remain unchanged in Figure 1b, and so do not contain deuterium. The deuterium distribution in mole per cent as calculated from molecular ion intensities in Figure 1b is: $D_0 = 38\%$, $D_1 = 7\%$, $D_2 = 55\%$, $D_3 = 0\%$. The ion of *m/e* 315 in Figure 1b (intensity 20.6% of *m/e* 314) is completely accounted for by the natural abundance of heavy isotopes associated with ions of mass 312, 313, and 314.[3] Peaks corresponding to M − 29, M − 31, and M − 43 are seen to contain deuterium, as expected from their identity (Table II). Of particular interest are the ions of mass 171 and 199 (Figure 1a) formed by cleavage of bonds on either side of C-10, with the positive charge retained on the oxygen-containing portions of the molecule (Ryhage and Stenhagen, 1960). In the spectrum of the labeled compound *m/e* 171 remains unchanged, but *m/e* 199 shifts partially to *m/e* 201, indicating the location of the two deuterium atoms to be at C-10 and the attached methyl group as expected.

Ergosterol. The mass spectrum of ergosterol has been previously reported (Friedland *et al.*, 1959; Fitches, 1963) but has not been studied in detail. However, mass spectra of ergosterol isolated from *N. crassa* grown in the presence of labeled and unlabeled methionine (Figure 2) permit conclusions regarding the number and location of deuterium atoms incorporated into the molecule. The only peaks of significant intensity are observed at *m/e* values greater than 250. Certain peaks (*m/e* 251,

[3] For a discussion of the calculation of heavy isotope distributions in organic molecules, see Biemann (1962).

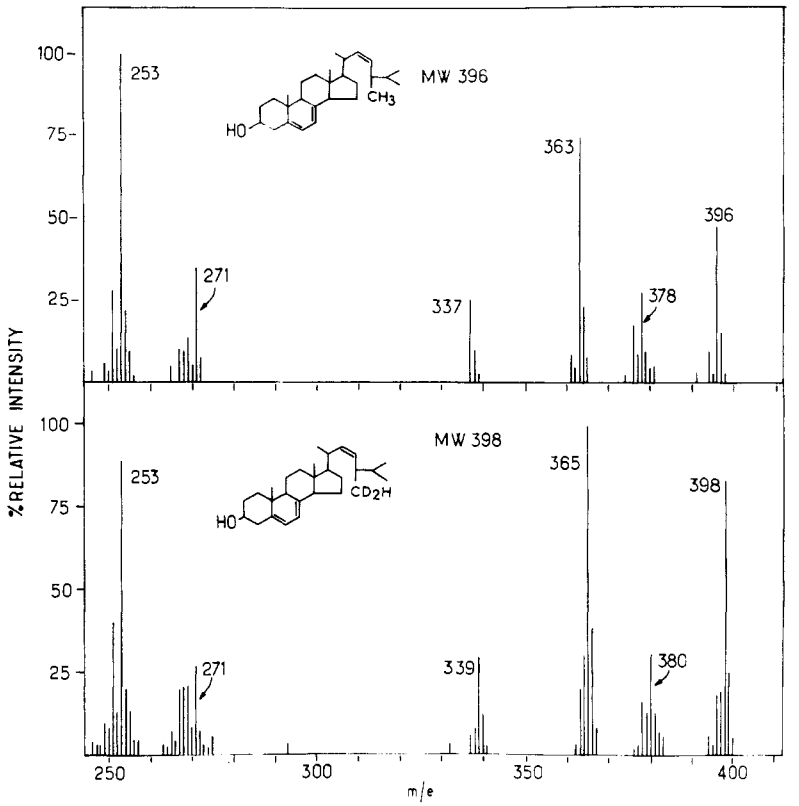


FIGURE 2: Mass spectra of ergosterol. Upper spectrum (2a), compound from cells grown on nonisotopic methionine. Lower spectrum (2b), compound from cells grown on methionine-methyl-d_3.

267–269, 376, 394) are associated principally with pyrolysis rather than electron impact–induced fragmentation, as indicated by the increase in their intensity with time, relative to the remainder of the spectrum.

The principal peaks in Figure 2a correspond to two general fragmentation paths, the first of which involves retention of the side chain at C-17, as shown in the scheme of Figure 3. The elimination of water from the molecular ion may also involve a hydrogen on C-2; however the C-3, C-4 process indicated in Figure 3 is favored because of the resulting stabilization gained from conjugation. Likewise, the methyl radical which is lost is shown as C-19, which yields an ion (*m/e* 363) well stabilized by the conjugated system in ring B. The sequence $378^+ \rightarrow 363^+$ is further indicated by a metastable peak at *m/e* 348.6 (calcd, 348.6). Thus, comparison of *m/e* 396, 378, and 363 in Figure 2a with the corresponding peaks in Figure 2b shows a shift of two mass units, owing to the incorporation of two deuterium atoms, to *m/e* 398, 380, and 365. The mole per cent of each species as calculated from the molecular peak is $D_0 = 16\%$, $D_1 = 13\%$, $D_2 = 71\%$, $D_3 = 0\%$. The natural abundance of heavy isotopes associated with the ions of mass 396, 397, and 398 completely accounts for *m/e* 399 in Figure 2b, thus showing the absence of any D_3 species.

Those peaks which remain unchanged in Figure 2b in comparison to Figure 2a (e.g., *m/e* 271, 253) correspond to a loss of the side chain from C-17, as shown in the scheme in Figure 3. The origin of *m/e* 253 from *m/e* 271 is indicated by a metastable peak at *m/e* 236.6 (calcd, 236.2).

It is of interest to note the absence of a peak of significant intensity at *m/e* 125 (Friedland *et al.*, 1959; Fitches, 1963), which would correspond to charge retention on the allylically stabilized side chain following rupture of the C-17, C-20 bond. Although its formation undoubtedly occurs to some extent, rapid decomposition to lower mass fragments would preclude its existence long enough to be collected and recorded. The mechanism of formation of *m/e* 339 in Figure 2b (*m/e* 337, Figure 2a), which contains two deuterium atoms, cannot be positively identified without specific deuterium labeling in rings A or B. However, since it contains the side chain it most likely involves elimination of carbon atoms 1, 2, 3, and the hydroxyl group, plus one rearranged hydrogen from C-19 or C-9.

Discussion

In these experiments, where the goal has been to determine the extent of the incorporation of deuterium from methionine-methyl-d_3 into the products, it was desirable to prevent dilution of the labeled methionine with nonisotopic methionine produced by biosynthesis in the organisms. It has been shown with *Escherichia coli* (Rowbury and Woods, 1961) and with *Saccharomyces cerevisiae* (Pigg *et al.*, 1962) that high concentrations of

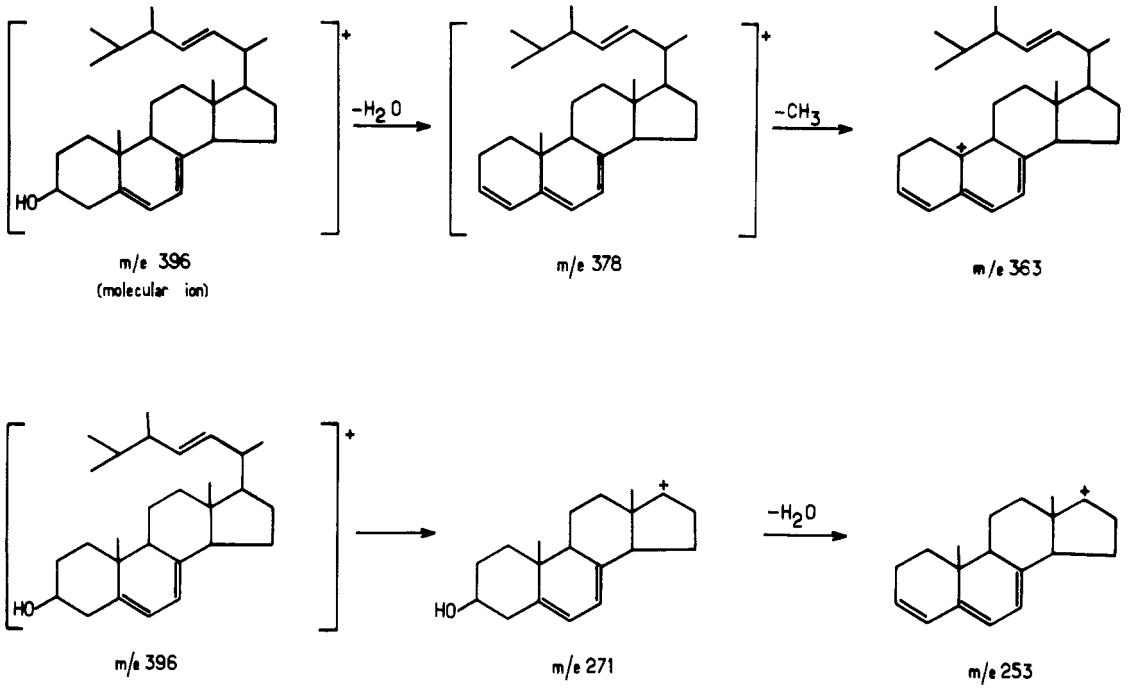


FIGURE 3: Fragmentation schemes for ergosterol.

methionine in the growth medium suppress the biosynthetic production of methionine by these organisms. We therefore employed fairly high levels of methionine-methyl-d_3 in the growth medium in the hope of effecting a similar suppression with *M. smegmatis*. This was only partially successful, since the organisms produced undeuterated products, by using endogenous non-isotopic methionine.

The most striking feature of the mass spectra of the deuterated products is the complete absence of any trideuterated species. Clearly, a proton is lost from the methyl group during some stage of its transfer from methionine to the final product. While the dideuterated species is the major product in the case of both tuberculostearic acid and ergosterol, the presence of a small amount of a compound which is presumably a monodeuterated species was observed in each case.

The possibility exists that methionine may be metabolized to other one-carbon compounds (e.g., formate, formaldehyde) which then either are more direct precursors of the methyl branches or are recycled to methionine with the loss of one or more protons. The results of Alexander *et al.* (1958) and of Parks (1958) make it unlikely that any one-carbon donor other than methionine and *S*-adenosylmethionine is involved in ergosterol synthesis. While some methionine may be catabolized, methyl groups which have passed through the catabolic process probably make a minor contribution to the final products compared with the contribution of the methionine originally present in the medium. However, if some methyl groups were oxidized to the formaldehyde level, it is possible that they would be further oxidized to the formate level (i.e., formate-d_1) before entering the route to methionine methyl groups again. Rachele *et al.* (1964) have recently shown that formaldehyde-d_2 gives rise to methionine-methyl-d_1 in the rat. Such a route, if present in *Mycobacterium* and *Neurospora*, could explain the presence of the small amounts of the D_1 products, but this route would make no contribution to the D_2 products. The possibility of simply reversing methionine synthesis to the methylene tetrahydrofolic acid stage before transfer of the one-carbon group is very unlikely to occur in growing cells, and Kisliuk (1963) has shown that this process does not occur *in vitro*.

It is reasonable to assume that in the processes leading from methionine to tuberculostearic acid and ergosterol the first step is the activation of methionine to *S*-adenosylmethionine. Sulfonium compounds of this type are capable of exchanging protons with the medium under certain conditions (Cotton *et al.*, 1959). One could propose that the results obtained in these experiments simply reflect loss of deuterium from *S*-adenosylmethionine by exchange with protons from water. This is not in accord with experiments in several other biological systems in which the methyl group of methionine maintained its integrity during activation and transfer (Keller *et al.*, 1949; duVigneaud *et al.*, 1956; Dewey *et al.*, 1954; Byerrum, *et al.*, 1954; Sato, *et al.*, 1957; Tropp, *et al.*, 1964; Jauréguiberry *et al.*, 1964a). Furthermore, it does not seem probable that exactly one proton would be lost by an exchange process, leaving no D_3 species and very little D_1 species.

It seems likely that the transalkylation processes which lead to tuberculostearic acid and ergosterol involve some common type of intermediate which,

because of its nature, must lose a proton. At present one can only guess as to what sort of compound this might be.[4]

Acknowledgment

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References

Alexander, G. J., Gold, A. M., and Schwenk, E. (1957), *J. Am. Chem. Soc. 79*, 2967.

Alexander, G. J., Gold, A. M., and Schwenk, E. (1958), *J. Biol. Chem. 232*, 599.

Alexander, G. J., and Schwenk, E. (1957), *J. Am. Chem. Soc. 79*, 4554.

Alexander, G. J., and Schwenk, E. (1958), *J. Biol. Chem. 232*, 611.

Biemann, K. (1962), Mass Spectrometry, New York, McGraw-Hill, chap. 5.

Birch, A. J., Eliot, D., and Penfold, A. R. (1954), *Australian J. Chem. 7*, 169.

Birch, A. J., English, R. J., Massy-Westropp, R. A., Slaytor, M., and Smith, H. (1957), *Proc. Chem. Soc.*, 204.

Birch, A. J., English, R. J., Massy-Westropp, R. A., Slaytor, M., and Smith, H. (1958), *J. Chem. Soc.*, 365.

Brodie, A. F., and Gray, C. T. (1956), *J. Biol. Chem. 219*, 853.

Byerrum, R. U., Flokstra, J. H., Dewey, L. J., and Ball, C. D. (1954), *J. Biol. Chem. 210*, 633.

Cotton, F. A., Fassnacht, J. H., Horrocks, W. D., Jr., and Nelson, N. A. (1959), *J. Chem. Soc.*, 4138.

Dewey, L. J., Byerrum, R. U., and Ball, C. D. (1954), *J. Am. Chem. Soc. 76*, 3997.

Fitches, H. J. M. (1963), *Advan. Mass Spectrometry, Proc. Conf. 2nd, Oxford, 1961*, 428.

Fleissner, E., and Borek, E. (1962), *Proc. Natl. Acad. Sci. U.S. 48*, 1199.

Fleissner, E., and Borek, E. (1963), *Biochemistry 2*, 1093.

Friedland, S. S., Land, G. H., Jr., Longman, R. T., Train, K. E., and O'Neal, M. J., Jr. (1959), *Anal. Chem. 31*, 169.

Gold, M., Hurwitz, J., and Anders, M. (1963), *Proc. Natl. Acad. Sci. U.S. 50*, 164.

Horowitz, N. H. (1947), *J. Biol. Chem. 171*, 255.

Jauréguiberry, G., Farrugia-Fougerouse, G., Audier, H., and Lederer, E. (1964a), *Compt. Rend. 259*, 3108.

Jauréguiberry, G., Law, J. H., McCloskey, J. A., and Lederer, E. (1964b), *Compt. Rend. 258*, 3587.

Keller, E. B., Rachele, J. R., and Vigneaud, V. du (1949), *J. Biol. Chem. 177*, 733.

Kisliuk, R. L. (1963), *J. Biol. Chem. 238*, 397.

Lederer, E. (1964a), *Biochem. J. 93*, 449.

Lederer, E. (1964b), *Experientia 20*, 473.

Lennarz, W. J., Scheuerbrandt, G., and Bloch, K. (1962), *J. Biol. Chem. 237*, 664.

Liu, T.-Y., and Hofmann, K. (1962), *Biochemistry 1*, 189.

Melville, D. B., Rachele, J. R., and Keller, E. B. (1947), *J. Biol. Chem. 169*, 419.

Mudd, S. H., and Cantoni, G. L. (1964), *in* Comprehensive Biochemistry, Florkin, M., and Stotz, E. H., eds., New York, Elsevier, Vol. XV, p. 1.

Parks, L. W. (1958), *J. Am. Chem. Soc. 80*, 2023.

Pigg, C. J., Spence, K. D., and Parks, L. W. (1962), *Arch. Biochem. Biophys. 97*, 491.

Pohl, S., Law, J. H., and Ryhage, R. (1963), *Biochim. Biophys. Acta 70*, 583.

Rachele, J. R., White, A. M., and Grünewald, H. (1964), *J. Biol. Chem. 239*, 353.

Rowbury, R. J., and Woods, D. D. (1961), *J. Gen. Microbiol. 24*, 129.

Rudney, H., and Parson, W. W. (1963), *J. Biol. Chem. 238*, 3137.

Ryhage, R., and Stenhagen, E. (1960), *Arkiv Kemi 15*, 291.

Ryhage, R., and Stenhagen, E. (1963), *in* Mass Spectrometry of Organic Ions, McLafferty, F. W., ed., New York, Academic, pp. 399–452.

Sato, C. S., Byerrum, R. U., and Ball, C. D. (1957), *J. Biol. Chem. 224*, 717.

Schlenk, F., and De Palma, J. R. (1957), *J. Biol. Chem. 229*, 1037.

Tropp, B. E., Law, J. H., and Hayes, J. M. (1964), *Biochemistry 3*, 1837.

Vigneaud, V. du, Rachele, J. R., and White, A. M. (1956), *J. Am. Chem. Soc. 78*, 5131.

Zalkin, H., Law, J. H., and Goldfine, H. (1963), *J. Biol. Chem. 238*, 1242.

[4] For a discussion of possible intermediates, see Lederer (1964a,b).