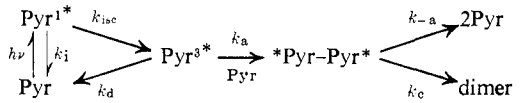
compounds compared to the rigidity of the pyrimidine rings.

(2) The values of k_a are quite large and resemble those one might estimate for the cyclic enones.[12] Triplet thymine adds to ground-state thymine only one-third as fast as triplet uracil adds to ground-state uracil. This effect is probably due to some steric hindrance by the methyl group of thymine.

(3) If k_d were the *only* pathway for radiationless decay, the rate constants in Table II and Lamola's Φ_{ISC} values would predict Φ_{DIM} values of 0.31 for 3.9×10^{-4} M uracil and 0.12 for 6.2×10^{-4} M thymine. There obviously is a further major source of inefficiency. The data demand that most of the original photoadduct of triplet base with ground-state base must be able to decay back to two ground-state molecules. The following mechanistic scheme yields eq 2 and 3, where ϕ_{AD} is the probablity that triplet base will react with ground-state base and ϕ_P is the probability that the intermediate will proceed on to stable dimer.



$$^1/_2\Phi_{\text{-PYR}} = \Phi_{DIM} = \left(\frac{k_{isc}}{k_i + k_{isc}}\right)\left(\frac{k_a[\text{Pyr}]}{k_d + k_a[\text{Pyr}]}\right)\left(\frac{k_c}{k_{-a} + k_c}\right) \quad (2)$$

$$\Phi_{DIM} = \Phi_{ISC}\phi_{AD}\phi_P \quad (3)$$

There are two possibilities for the structure of the intermediate: (1) a triplet excimer, or (2) a ground-state σ-bonded biradical. A singlet excimer does intervene in singlet-state dimerizations, but it proceeds on to stable ground-state dimer with 100% efficiency ($\phi_P = 1$).[13] The low ϕ_P values for triplet uracil and thymine, as well as their relative values, are nicely consistent with a biradical intermediate. Cleavage of 1,-4 biradicals is always an important reaction;[14] coupling of the bistertiary or secondary, tertiary biradical from thymine should be slower than coupling of the necessarily bissecondary biradical from uracil. Since all four *cis*-fused dimers may be formed,[9] the k_a and ϕ_P values we report are probably composites of four sets of such values. Consequently, until the actual structures of the triplet-state photodimers are determined, further speculation about the nature of the intermediate would be meaningless.

Toki and Sakurai have proposed a very similar scheme, based on similar kinetic studies, to explain the low quantum efficiency for the photocycloaddition of benzophenone to furan.[15] No triplet-state photocycloaddition yet reported proceeds with unit quantum yield, even extrapolated to infinite substrate concentration. It is likely that reversible adduct formation occurs in all cases, especially if biradicals are involved.

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(16) Fellow of the Alfred P. Sloan Foundation, 1968–1970.
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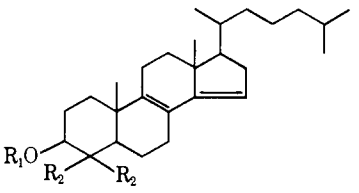
Evidence for the Biological Conversion of $\Delta^{8,14}$ Sterol Dienes into Cholesterol

Sir:

As previously reported,[1] the elimination of the 14α-methyl group of lanosterol during its biological conversion into cholesterol is accompanied by the stereospecific removal of one of the hydrogen atoms in position 15. Correct interpretation of stereochemical requirements in the formation of farnesyl pyrophosphate[2] from *dl*-(2*S*)-[2-^{3}H]mevalonic acid shows that the labeled hydrogen atoms are present in positions 1α, 7β, 15α, 22*S*, 26 or 27, and 30 or 31 of lanosterol.[3] Our previous results show that the hydrogen eliminated is the one at position 15α and not position 15β as erroneously stated.

Our results allowed us to hypothesize the existence of not yet recognized intermediates between lanosterol[4] and 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol[5] in the biosynthetic pathway to cholesterol. The saturation of the double bond in the side chain is known to occur at different stages.[6] A possible precursor appeared to be 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**1**), and this hypothesis has been verified by studying the transformation of the labeled compound into cholesterol in rat liver homogenates.



1, $R_1 = H$; $R_2 = CH_3$
2, $R_1 = COCH_3$; $R_2 = CH_3$
3, $R_1 = H$; $R_2 = H$
4, $R_1 = COCH_3$; $R_2 = H$

Radioactive **1**[5,7] (8.58 μCi/μmol) was prepared as described for 5α-cholesta-8,14-dien-3β-ol[8] by isomerization of 4,4-dimethyl-cholesta-5,7-dien-3β-ol[9] in the

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(7) The chemical purity of all compounds was established by comparing melting points, optical rotation values, mass spectra, and glpc retention times on a 1% phenylsilicone glass column with those of authentic samples. The radioactive purity of the precursors was established by determining the distribution of radioactivity on thin layer (kieselgel G–silver nitrate, 100:30) developed with chloroform. At least 98% of the initial radioactivity was found in the spot corresponding to assayed compound.
(8) L. F. Fieser and G. Ourisson, *J. Am. Chem. Soc.*, **75**, 4405 (1953).

presence of HTO (0.5 Ci/g). The positions of the tritium atoms were inferred from the mass spectrum of the deuterated compound **2** obtained in the presence of DCl in D_2O and O-deuteriomethanol. The mass spectrum of **2** (Figure 1a) showed prominent peaks at *m/e* 454 (M), 439 (M − CH_3), 394 (M − CH_3COOH), 379 (M − CH_3COOH − CH_3), 341 (M − R), and 281 (M − CH_3COOH − R, where R = C_8H_{17}, the alkyl side chain of the sterol). The peaks described above were shifted up to five mass units higher in the spectrum of the deuterated compound **2** (Figure 1b). The spectrum also indicates that the five deuterium atoms are in the rings.

Compound **1** (1.02 μCi) was incubated[10] under anaerobic conditions with rat liver homogenate,[11] and the unsaponifiable residue (0.86 μCi; 85% of total radioactivity) from the homogenate was acetylated. Carrier 4,4-dimethyl-5α-cholest-8-en-3β-ol acetate[5] was added and the mixture was separated by silver nitrate–kieselgel G–Celite column chromatography[12] into four main fractions corresponding to:[7] (a) 4,4-dimethyl-5α-cholest-8-en-3β-ol acetate (0.536 μCi; 61.8% of unsaponifiable radioactivity); (b) a mixture of 4,4-dimethyl-5α-cholest-8-en-3β-ol acetate and cholesteryl acetate (0.07 μCi; 8.1%); (c) cholesteryl acetate (0.001 μCi; 0.1%); (d) unchanged **1** as acetate (**2**) (0.228 μCi; 26.4%). Carrier cholesteryl acetate was added to fraction c and no radioactivity was found after two crystallizations. Carrier 4,4-dimethyl-5α-cholest-8-en-3β-ol acetate was added to fraction a and the mixture was crystallized three times to yield radioactive material (0.521 μCi; 52% of radioactivity added as compound **1**). The presence of 4,4-dimethyl-5α-cholest-14-en-3β-ol acetate,[13] mp 131°, M^+ 456 (prepared as described[14] for 5α-cholest-14-en-3β-ol acetate) in the radioactive material was excluded since under our conditions this sterol acetate was eluted from the columns after cholesteryl acetate. The presence of 4,4-dimethyl-5α-cholest-8(14)-en-3β-ol acetate[5] was also excluded since a portion of fraction a, obtained from another incubation experiment, diluted with this ester, saponified, and crystallized, showed the great loss of radioactivity reported by Gautschi and Bloch[5] (90% after three crystallizations).

Compound **1** (0.254 μCi) was incubated in an oxygen atmosphere, and the unsaponifiable residue (0.244 μCi; 98% of total radioactivity) from the homogenate was acetylated and separated by silver nitrate–kieselgel G–Celite column chromatography.[12] The fraction corresponding to pure cholesteryl acetate (0.121 μCi; 47.6% of unsaponifiable radioactivity) was diluted with inactive material and purified through the dibromide.[15] The recovered cholesteryl acetate (0.056 μCi) contained 22.2% of the total radioactivity.

These results indicate that compound **1** is converted into cholesterol by rat liver homogenates *in vitro* in the presence of oxygen, while under anaerobic conditions the conversion is stopped at the level of C_{29} sterol monoenes. In aerobic conditions the conversion of compound **1** into cholesterol is certainly higher than indicated by the radioactivity value, because during the conversion a certain amount of radioactivity must be lost from positions 5 and/or 6 and/or 7.[16,17]

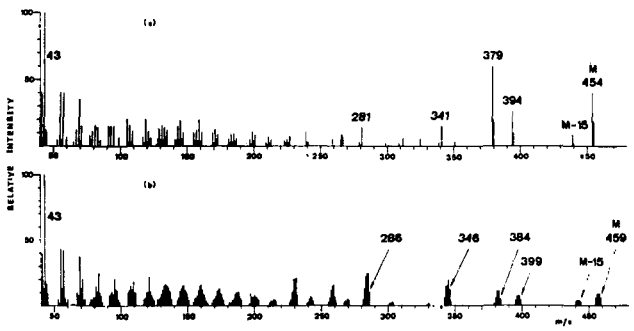

Figure 1. Mass spectrum of (a) 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol acetate and (b) deuterated 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol acetate (LKB 9000, 70 eV, PhSi 1% column, 240°).

Since it is known[6] that isomerization of the 8,9 double bond and saturation of the 24,25 double bond occur at the level of various biosynthetic intermediates, it was hypothesized that saturation of the 14,15 double bond was not a characteristic reaction of compound **1** alone.

5α-Cholesta-8,14-dien-3β-ol[8] (**3**) was oxidized by the method of Oppenauer to 5α-cholesta-8,14-dien-3-one:[13] mp 133°; uv max (C_2H_5OH) 252 mμ (ε 17,200). This ketone was labeled at positions 2 and 4 with HTO,[18] and its reduction with $LiAlH_4$ yielded radioactive 5α-cholesta-8,14-dien-3β-ol[7] (**3**) (6.3 μCi/μmol). Compound **3** (0.77 μCi) was incubated under anaerobic conditions with rat liver homogenate and the unsaponifiable residue from the homogenate was acetylated. Carrier 5α-cholest-7-en-3β-ol, which is the biological transformation product of 5α-cholest-8-en-3β-ol,[6] was added as the acetate. The mixture was separated by silver nitrate–kieselgel G–Celite column chromatography into four main fractions corresponding to:[7] (a) 5α-cholest-7-en-3β-ol acetate (0.098 μCi; 12.8% of total radioactivity); (b) a mixture of 5α-cholest-7-en-3β-ol acetate and cholesteryl acetate (0.076 μCi; 9.9%); (c) cholesteryl acetate (less than 0.1%); (d) unchanged **3** as acetate (**4**) (0.208 μCi; 27%). Fraction a was diluted again with inactive carrier and transformed into 5α-cholestane-3β,7,8-triol 3β-acetate[16] (0.05 μCi; 6.5% of total radioactivity). This result shows the ability of the biological system to saturate the 14,15 double bond of a $\Delta^{8,14}$ diene not only in C_{29} but also in C_{27} sterols.

The high incorporation of 4,4-dimethyl-5α-cholesta-8,14-dien-3β-ol (**1**) supports the hypothesis that sterols containing the $\Delta^{8,14}$ diene system are among the biological precursors of cholesterol. To our knowledge, these compounds have not yet been identified in biological tissues, suggesting that they may be very rapidly

(9) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1131 (1957).
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metabolized. Experiments are now being carried out in order to establish the biological role of the $\Delta^{8,14}$ sterol dienes.

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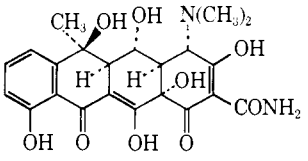
Tetracyclines. VII. Total Synthesis of *dl*-Terramycin[1]

Sir:

Terramycin is one of the most important broad-spectrum antibiotics used in medicine today. It was the first member within the family of tetracycline antibiotics to have its structure fully elucidated in the laboratories of Chas. Pfizer & Co., Inc., in close cooperation with Woodward.[2] Structure and configuration **1** for this compound have been confirmed by X-ray analysis[3,4] and by nmr analysis.[5]



1

Because Terramycin (**1**) is one of the most highly substituted and chemically labile members of the tetracycline family, its synthesis has remained an intriguing and challenging problem. We now wish to report the first synthesis of this compound as its racemate.[6,7] This synthesis is another example of a general method[8] for synthesizing tetracyclines of both known and novel structures.

(1) Terramycin is a registered trademark of Chas. Pfizer & Co., Inc. for oxytetracycline.

(2) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Ragna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **75**, 5455 (1953).

(3) Y. Takeuchi, and M. J. Buerger, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1366 (1960).

(4) H. Cid-Dresdner, *Z. Kristallogr.*, 121, 170 (1965).

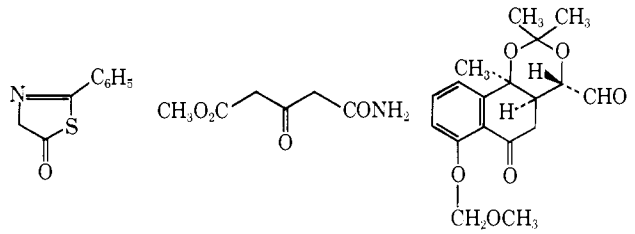
(5) M. Schach von Wittenau, R. K. Blackwood, L. H. Conover, R. H. Glauert, and R. B. Woodward, *J. Am. Chem. Soc.*, **87**, 134 (1965).

(6) The simplest compound deriving from a fermentation product and having full antibacterial activity is 6-demethyl-6-deoxytetracycline. This compound was first synthesized by J. J. Korst, J. D. Johnston, K. Butler, E. J. Bianco, L. H. Conover, and R. B. Woodward, *ibid.*, **90**, 439 (1968) [preliminary reports: (a) L. H. Conover, K. Butler, D. Johnston, J. J. Korst, and R. B. Woodward, *ibid.*, **84**, 3222 (1962); (b) R. B. Woodward, *Pure Appl. Chem.*, **6**, 561 (1963)]. Another synthesis of this compound was reported later.[8]

(7) A synthesis of 12a-deoxy-5a,6-anhydrotetracycline has recently been reported: A. I. Gurevich, M. G. Karapetyan, M. N. Kolosov, V. G. Korobko, S. A. Popravko, and M. M. Shemyakin, *Tetrahedron Letters*, 131 (1967). Syntheses of other tetracyclic compounds deriving from tetracyclines are summarized by H. Muxfeldt and R. Bangert, *Progr. Chem. Org. Nat. Products*, 21, 116 (1963).

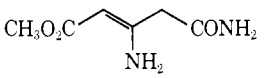
(8) H. Muxfeldt and W. Rogalski, *J. Am. Chem. Soc.*, **87**, 933 (1965).

Terramycin (**1**) was assembled from three basic building blocks: the thiazolone **2**, methyl 3-oxoglutaramate (**3**), and the aldehyde **4**. The preparation of



2 **3** **4**

the thiazolone **2** has been described recently.[9] Methyl 3-oxoglutaramate (**3**) (mp 36–38°; λ_{max} mμ (ϵ) 273 (17,500) in 0.01 *N* NaOH; λ_{max} μ 5.75, 5.80, 5.95, and 6.30 in $CHCl_3$) was obtained by acid hydrolysis of the enamine **5** (mp 120–121°; λ_{max} mμ (ϵ) 276 (16,600) in MeOH; λ_{max} μ 5.96, 6.18, and 6.40 in $CHCl_3$). Enamine **5** was prepared by carefully controlled treatment of dimethyl 3-oxoglutarate with ammonia in methanol.



5

The synthesis of aldehyde **4** has already been published in part.[10] Starting material was the diene adduct **6** of juglone acetate and 1-acetoxybutadiene. This compound was converted over seven steps in high yield into the aldehyde **7**. Ozonolysis followed by hydrolysis of the crystalline ozonide yielded a crystalline mixture of **8** and **9**. Aqueous sodium carbonate cleaved these substances in 85% yield to a mixture of aldehydes **10** and **11** (melting range 120–160°). The pure isomers, mp 140–143° and 171–173°, respectively, could be obtained. That the higher melting aldehyde is aldehyde **11** was deduced from its nmr spectrum (H_{4a}, δ 9.60, d, J = 1.5 Hz; H_5, δ 4.06, dd, J = 1.5 and 11.5 Hz; H_{5a}, δ 2.43, dt, J = 11.5 and 4.0 Hz; 11a protons, δ 2.98, d, J = 4.0 Hz; in $CDCl_3$).[11] Cleavage of the mixture of **8** and **9** in deuterium oxide with sodium carbonate to aldehydes **12** and **13** further confirmed that in **11** and **13** epimerization at C-5 had occurred. Aldehyde **13** had incorporated deuterium at C-5 as evidenced by the nmr spectrum (H_{4a}, δ 9.60, s; H_{5a}, δ 2.42, s; in $CDCl_3$).

The desired aldehyde **4** could be easily prepared from the mixture of aldehydes **10** and **11** by a three-step procedure. Piperidine in refluxing benzene converted the aldehydes to **14** (91%; mp 118–119°; λ_{max} μ 5.97 and 6.10 in $CHCl_3$). This enamine was alkylated with chloromethyl methyl ether *via* its sodium salt to **15** (90%; mp 81–84°; λ_{max} μ 5.96 and 6.28 in $CHCl_3$). When **15** was adsorbed on deactivated silica gel, selective hydrolysis of the enamine function occurred, and the oily aldehyde **4** was formed (72%). This hydrolysis was stereospecific since **4** had an nmr spectrum consistent only with a *trans* coplanar relationship of the hydrogens at C-5 and C-5a (H_{5a}, δ 9.59, d, J = 1.0 Hz; H_5, δ 4.11, dd, poorly resolved, J = 1 and 11.5 Hz; in $CDCl_3$). Furthermore the aldehyde **11** was regenerated

(9) H. Muxfeldt, J. Behling, G. Grethe, and W. Rogalski, *ibid.*, **89**, 4991 (1967).

(10) H. Muxfeldt, *Angew. Chem.*, 74, 825 (1962). The melting points of 10 and 11 are interconverted in this paper.

(11) Numbering as in Terramycin.