(9).[2-4] Such behavior has now been observed in hexaborane(10) also.

We wish to report the preparation of the new anion nonahydrohexaborate(−1), $B_6H_9^-$, through the following general reaction

$$B_6H_{10} + MB \longrightarrow MB_6H_9 + HB$$
$$MB = LiCH_3, NaH, KH$$

In a typical reaction, potassium hydride reacts with hexaborane(10) in an ether solvent at low temperature to liberate 1 mole of hydrogen per mole of $B_6H_{10}$. The resulting salt, $KB_6H_9$, is isolated as a crystalline solid which decomposes slowly at room temperature in a dry inert atmosphere. *Anal.* Calcd for $KB_6H_9$: B, 56.8; H, 8.03. Found: B, 57.4; H, 7.93.

The $B_6H_9^-$ anion appears to be significantly more stable, thermally, than its analog $B_5H_8^-$,[2-4] which is derived from $B_5H_9$. The boron-11 nmr spectrum of $KB_6H_9$ in THF persists for approximately 1 day at 30°, while the spectrum of $KB_5H_8$ under similar conditions is markedly deteriorated after several minutes.[4]

The boron-11 nmr spectrum of $KB_6H_9$ in THF is essentially independent of temperature in the range studied (−80 to 30°) with respect to appearance, chemical shifts, and coupling constants; it consists of two symmetrical doublets in an area ratio of 1:5, qualitatively identical with hexaborane(10). The smaller doublet ($\delta$ 48.3 ppm with respect to $BF_3 \cdot OEt_2$, $J$ = 134 Hz) is assigned to the apical boron in the framework. The doublet at lower field ($\delta$ −9.5 ppm, $J$ = 100 Hz) is assigned to basal borons which are apparently magnetically equivalent. The chemical shifts and coupling constants of the anion are significantly different from those of the parent compound.[5]

The reaction of $B_6H_9^-$ with DCl at low temperature regenerated hexaborane(10) in high yields (*ca.* 90%). The entering deuterium was found only in the bridge position as shown by the infrared spectrum. A band at 1137 $cm^{-1}$ was observed which does not occur in the spectrum of hexaborane(10) of normal isotopic composition.[6] It is assigned to the B–D–B stretch. The isotope shift ($\nu_{B-H-B}/\nu_{B-D-B}$ = 1.30) is in excellent agreement with the shift observed for the bridge system in pentaborane(9).[7] This result, plus the fact that the boron-11 nmr spectrum of the ion closely resembles that of the parent compound, indicates that the framework of the ion is the same as that of hexaborane(10), a pentagonal pyramid.

Deprotonation of $B_6H_{10}$ takes place with loss of a bridge hydrogen. Reaction of $CH_3Li$ with hexaborane(10) containing deuterium in terminal positions[8] on basal borons yielded only $CH_4$; no $CH_3D$ was observed in the mass spectrum. The boron-11 nmr spectrum of the resulting ion showed that the apical boron had retained its hydrogen. Therefore the proton which was removed must have come from the bridge position.

For the boron hydrides $B_5H_9$, $B_6H_{10}$, and $B_{10}H_{14}$, the experimental evidence shows that bridge hydrogens are more acidic with respect to deprotonation reactions than terminal hydrogens. It was of interest to determine the relative acid strengths of the series since it had been predicted that acid character increases as the polyhedral boron framework increases in size.[9] We have confirmed this order for the series cited above. From boron-11 nmr, the following acid–base reactions appear complete.

$$LiB_5H_8 + B_6H_{10} \longrightarrow B_5H_9 + LiB_6H_9 \quad (1)$$
$$LiB_6H_9 + B_{10}H_{14} \longrightarrow B_6H_{10} + LiB_{10}H_{13} \quad (2)$$

The first reaction was run in ethyl ether at −60°. The nmr spectrum recorded at −30° showed no trace of reactants. The second reaction, also in ethyl ether, proceeded below −78° as evidenced by the appearance of the bright yellow color characteristic of $B_{10}H_{13}^-$ in solution. The nmr spectrum at −10° showed only resonances attributable to $B_6H_{10}$ and $LiB_{10}H_{13}$.

Recent theoretical calculations of the ground-state charge distribution of diborane(6)[10-14] and the higher boron hydrides[11] indicate that the bridge hydrogens are more positive than terminal hydrogens.

**Acknowledgment.** We wish to acknowledge, gratefully, support of this research through Grants GP-7557X and GP-8321 by the National Science Foundation.

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## Separation of the Cyclization and Rearrangement Processes of Lanosterol Biosynthesis. Enzymic Conversion of 20,21-Dehydro-2,3-oxidosqualene to a Dehydroprotosterol

*Sir:*

Structure **1**, a cation or its functional equivalent having an enzymic or nonenzymic leaving group attached to C-20, is presumed to be the first tetracyclic intermediate in the enzymic cyclization of 2,3-oxidosqualene[1-3] and the starting point for the multi-

(1) (a) E. J. Corey and W. E. Russey, *J. Am. Chem. Soc.*, **88**, 4751 (1966); (b) E. J. Corey, W. E. Russey, and P. R. Ortiz de Montellano, *ibid.*, **88**, 4750 (1966); (c) W. E. Russey, Ph.D. Thesis, Harvard University, 1966.
(2) (a) E. E. van Tamelen, J. D. Willet, R. B. Clayton, and K. E. Lord, *J. Am. Chem. Soc.*, **88**, 4752 (1966); (b) J. D. Willet, K. B. Sharpless, K. E. Lord, E. E. van Tamelen, and R. B. Clayton, *J. Biol. Chem.*, **242**, 4182 (1967).
(3) For previous paper from this laboratory see E. J. Corey, P. R. Ortiz de Montellano, and H. Yamamoto, *J. Am. Chem. Soc.*, **90**, 6254 (1968).

group rearrangement[4] which leads to lanosterol. The biological lifetime of **1** in mammalian steroidogenesis is unknown, since such structures have not been detected as intermediates in the conversion of 2,3-oxidosqualene to lanosterol in the usual liver enzyme systems. Nor can a decision be reached at present as to whether the cyclization and rearrangement steps require two different enzymes. These and other problems of sterol biosynthesis led to the studies reported herein.

The cyclization of the unnatural substrate 20,21-dehydro-2,3-oxidosqualene (**2**) by a solubilized microsomal preparation containing 2,3-oxidosqualene–sterol cyclase[5] would be expected to generate the highly stabilized cation **3** (or its functional equivalent), from which rearrangement in the direction of lanosterol should be energetically unfavorable. In this event a dehydroprotosterol[3] should result and should be an isolable product. This expectation has now been confirmed by the finding that the *unrearranged* cyclization product **4** is formed by the action of 2,3-oxidosqualene–sterol cyclase on **2**.

Racemic tritium-labeled 20,21-dehydro-2,3-oxidosqualene (**2**), labeled at C-17 (4.9 $\mu$mol, specific activity $2.75 \times 10^5$ dpm/$\mu$mol), was incubated anaerobically with a crude solution of 2,3-oxidosqualene–sterol cyclase (250 ml in 0.1 *M* phosphate buffer containing 0.4 *M* potassium chloride at pH 7.5 as prepared from *ca.* 60 g of hog liver microsomes by the desoxycholate method of solubilization[5]) in the presence of 10 mg of Tween 80 at 32° for 5 hr, and the extracted non-saponifiable lipid (1.27 g) was subjected to thin layer chromatographic (tlc) purification on neutral alumina under an argon atmosphere (solvent, $CH_2Cl_2$ containing a trace of hydroquinone) to remove the bulk of the microsomal lipid.[6] The major enzymic product obtained from the chromatographic separation in 14% yield (total radioactivity $1.9 \times 10^5$ dpm) had $R_f$ *ca.* 0.1 and was clearly distinguishable from the starting oxide **2** ($R_f$ 0.65, *ca.* 75% recovery) as well as either lanosterol ($R_f$ 0.45) or cholesterol ($R_f$ 0.3). The chromatographic mobility of this enzymic product is in accord with expectations based on the diol formulation **4** which is proposed on the basis of the data presented below. The enzymic product was found to be so sensitive to oxygen that complete purification by chromatography was not feasible and, consequently, characterization was carried out after selective oxidation designed to cleave the reactive side chain of **4**. Treatment of the enzymic product ($1.9 \times 10^5$ dpm, 305 $\mu$g) with potassium permanganate (8.1 mg) and potassium periodate (450 mg) in aqueous *t*-butyl alcohol at pH 8.5 for 3 hr at 25° afforded after tlc separation on alumina ($CH_2Cl_2$) 5 mg of steroidal material containing 180 $\mu$g, $1.35 \times 10^5$ dpm (71%), of labeled oxidation product, $R_f$ 0.25. After acetylation of this material using a large excess of acetic anhydride–pyridine, tlc purification (silica gel, $CH_2Cl_2$, $R_f$ 0.30), and sublimation *in vacuo*, a product to which is assigned structure **5**, R = Ac, was obtained as fine colorless crystals (100 $\mu$g), >90% pure by tlc and gas chromatographic (gc) analysis.[7] The nmr spectrum of this product, obtained in $CCl_4$ at 100 MHz using computer time averaging over 36 scans for signal enhancement, showed two 3-H singlets due to $CH_3CO$ at 2.16 and 2.22 ppm and five 3-H singlets at 0.81, 0.99, 1.06, 1.08, and 1.17 ppm, as expected for the five angular methyl groups in structure **5**, R = Ac. The mass spectrum,[8] which was also in accord with structure **5**, R = Ac, showed the molecular ion at *m/e* 402 and the expected major fragments corresponding to loss of $CH_3CO_2H$ (*m/e* 342) and $CH_3CO_2H + CH_3CO$ (*m/e* 299), with a weaker peak due to loss of $CH_3CO_2H$ and $CH_3$ (*m/e* 327). All the other peaks in the spectrum above *m/e* 70 having an intensity >10% of the strongest peak ($CH_3CO^+$ at *m/e* 43) can be accounted for very simply on the basis that these arise from cleavage of the common ancestor **6** into pairs of fragments, each of which is detected as an ion. Table I summarizes the observed peaks corresponding to fragment pairs and the bond fission required to generate them. Plausible mechanisms for these cleavages can be derived.

**Table I.** Mass Spectral Peaks from Cleavage of **6** (Originating from **5**, R = Ac) into Pairs of Ions

| Fragment pair, *m/e*[a,b] | Required C–C bond (and ring) cleavage |
|---|---|
| 217, 81 | 8,14 and 12,13 (C) |
| 205, 95 | 8,14 and 11,12 (C) |
| 191, 109 | 8,14 and 9,11 (C) |
| 147, 149, 151 | 9,10 and 7,8 (B) |
| 135, 163 | 9,10 and 6,7 (B) |
| 121, 175 | 9,10 and 5,6 (B) |

[a] In most cases two or three peaks separated by 2 *m/e* units appeared together as a multiplet; only the strongest peak in each multiplet is recorded here. [b] The first *m/e* value on each line corresponds to the fragment containing the A ring and the second to the fragment containing the D ring.

The saturated, tetracyclic formulation **5** is also supported by chemical evidence including inertness to osmium tetroxide under drastic conditions. Oxidation of **5**, R = H, with excess alkaline sodium hypoiodite reagent produced an acidic product **7**, R′ = H, which was converted by treatment with diazomethane to a neutral compound, the methyl ester **7**, R′ = $CH_3$, isolated by tlc (silica gel, 20% ethyl acetate in benzene, $R_f$ 0.4). The mass spectrum[8] of the ester was in full accord with structure **7**, R′ = $CH_3$, and displayed peaks due to elimination of functionality at *m/e* 361 ($-CH_3$), 358 ($-H_2O$), 345 ($-OCH_3$), 343 ($-H_2O$, $-CH_3$), and 327 ($-H_2O$, $-OCH_3$), in addition to the molecular ion at 376, and peaks due to cleavage at the B or C ring similar in origin to those observed for the keto acetate **5**, R = $CH_3CO$, which are listed in Table I.

The enzymic formation of the tetracyclic diol **4** could be completely inhibited by the addition of 2,3-iminosqualene[9] to solutions of 2,3-oxidosqualene–sterol

(4) R. B. Woodward and K. E. Bloch, *J. Am. Chem. Soc.*, 75, 2023 (1953). See also J. W. Cornforth, *Angew. Chem. Intern. Ed. Engl.*, 7, 903 (1968).

(5) P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch, and E. J. Corey, *J. Biol. Chem.*, 242, 3014 (1967); P. R. Ortiz de Montellano, Ph.D. Thesis, Harvard University, 1968, p 106.

(6) No effort was made to remove microsomal lipid materials from the enzyme solution.

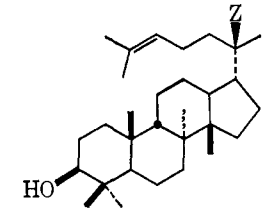
(7) Vpc analysis was carried out using a 10-ft, 0.125-in. column of 2% OV-1 (Supelco Inc.) on silanized support (Gaschrome Q) at 250° and a $N_2$ flow rate of 60 cc/min. The product had a retention time ($t_r$) of 10.1 min.

(8) Obtained using a mass spectrometer (LKB Instrument Co.) coupled to a gc apparatus fitted with an OV-1 column. We are indebted to Dr. James Orr, Massachusetts General Hospital, for these data.

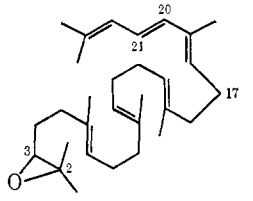
(9) E. J. Corey, P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean, *J. Am. Chem. Soc.*, 89, 2797 (1967).

cyclase and 20,21-dehydro-2,3-oxidosqualene (**2**) which otherwise produced **4**. In addition, the rate of conversion of 2,3-oxidosqualene to lanosterol under anaerobic conditions with 2,3-oxidosqualene–sterol cyclase was decreased by the addition of the substrate **2**, *e.g.*, the rate of formation of lanosterol was depressed by *ca.* 30% when a mixture of 2,3-oxidosqualene and 5 molar equiv of **2** was incubated with 2,3-oxidosqualene–sterol cyclase. These results clearly indicate the involvement of this enzyme in the transformation of **2** to the cyclization product **4**. The stereochemistry of **4** can reasonably be assumed as shown on this basis.[10]

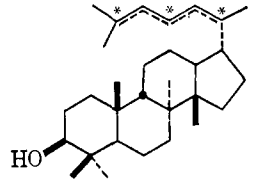
The synthesis of 20,21-dehydro-2,3-oxidosqualene (**2**) was accomplished from the aldehyde **8**[11] which was prepared by treatment of squalene with 1 equiv of ozone at −78° in methylene chloride solution, reduction with zinc–acetic acid, and purification by chromatography followed by fractional distillation. Reaction of **8** with N-bromosuccinimide–water–glyme afforded after tlc purification the bromohydrin corresponding to the addition of HOBr to the double bond farthest removed from the formyl group. This bromohydrin (10 mg) was labeled with tritium at the carbon $\alpha$ to the carbonyl by exposure to a mixture of tritiated water (10 $\mu$l, 1 mCi/mg) and tetrahydrofuran–triethylamine (0.5 ml, 18:1) at 50° for 14 hr. Reaction of the labeled or unlabeled bromohydrin with an excess of the Wittig reagent from triphenyl-6-methylhepta-3(*trans*),5-dien-2-ylphosphonium bromide and lithium diisopropylamide in tetrahydrofuran at 0° furnished 20,21-dehydro-2,3-oxidosqualene (**2**).[11,12]



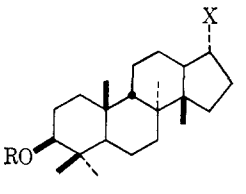
**1**, Z = +, enzymic or nonenzymic functional group



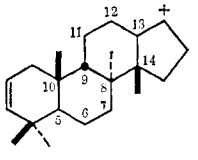
**2**



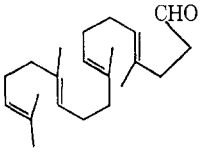
**3**, $\delta^+$ at C*
**4**, HO at one C*



**5**, X = $CH_3CO$
**7**, R = H; X = COOR'



**6**, *m/e* 299



**8**

(10) A correlation of **4** with a naturally occurring protosterol derivative of known structure and stereochemistry [see S. Okuda, Y. Sato, T. Hattori, and H. Igarashi, *Tetrahedron Letters*, 4769 (1968)] will be reported in due course.

(11) Satisfactory spectroscopic (ir, nmr, mass) and analytical data were obtained for this product.

(12) The phosphonium salt was prepared from triphenylphosphonium bromide and 6-methylhepta-3(*trans*),5-dien-2-ol [J. Colonge and J. Varagnat, *Bull. Soc. Chim. France*, 1220 (1961)] in tetrahydrofuran at 25° for 20 hr.

A previous study,[3] closely related to that reported here, dealt with the enzymic formation of a bisnorprotosterol derivative lacking the methyl substituents at C-8 and C-14. The two systems now known which allow separation of the cyclization and rearrangement steps of mammalian steroidogenesis afford opportunities for the investigation of these steps in more detail. For example, reaction of the labeled ketone **5**, R = H, with 4-methylpent-3-enylmagnesium bromide affords the labeled protosterol **1**, Z = OH, an intermediate of great interest which is currently under investigation.[13]

(13) This work was supported by the National Institutes of Health, the National Science Foundation, and the Hoffmann-La Roche Co.

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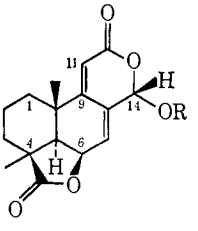
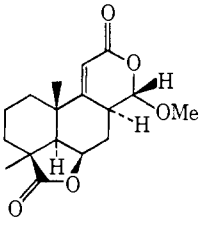
## Structure of a $C_{17}$ Antifungal Terpenoid from an Unidentified Acrostalagmus Species

*Sir:*

We report on the structure of a new antifungal[1] and biogenetically significant mold metabolite, LL-Z1271$\alpha$ (I), mp 214–216°, $[\alpha]_D$ −203° (*c* 0.29, MeOH), obtained from an unknown Acrostalagmus species known as culture LL-Z1271 in these laboratories. A minor metabolite is shown to be the corresponding lactol, LL-Z1271$\gamma$ (II), mp 238–240°, $[\alpha]_D$ −259° (*c* 0.52, MeOH).



I, R = Me
II, R = H
III, R = Ac



IV

Mass spectral and elemental analysis[2] indicated the molecular formula of I to be $C_{17}H_{20}O_5$. The 60-Mc nmr spectrum showed the presence of two tertiary C-methyl's ($\delta$ 1.16 and 1.33) and a methoxy group at $\delta$ 3.70 in addition to four deshielded protons which from their chemical shifts must be attached to double bonds and carbon atoms bearing oxygen. The infrared spectrum suggested the presence of a $\gamma$-lactone (1775

(1) I possessed significant antifungal activity *in vitro* against a number of fungi and *in vivo* against some experimental ringworm infections in guinea pigs.

(2) Satisfactory analyses (elemental or mass spectral or both) were obtained for all compounds reported; ultraviolet spectra were taken in methanol and are reported in m$\mu$ ($\epsilon$); infrared spectra were taken in KBr pellets and are reported in $cm^{-1}$ for the hydroxyl and carbonyl regions only. Nmr spectra were measured at 60 and 100 Mc in deuteriochloroform (unless otherwise stated) with in some cases a small amount of DMSO added; shifts are expressed as $\delta$ values (parts per million) from tetramethylsilane as internal standard and coupling constants (*J*) are expressed in cycles per second (Hz). We thank W. Fulmor and L. Brancone and associates for the spectral and analytical data, Dr. J. Lancaster of the Stamford Laboratories for the spin-decoupling experiments, and Drs. J. Karliner and G. Van Lear for the mass spectra. We also thank A. C. Dornbush and G. S. Redin and associates for the *in vitro* and *in vivo* antifungal testing, Dr. P. Shu and associates for the fermentations and initial processing, and Dr. H. Tresner for the identification of the culture.