studies on $Cu_2B_{10}H_{10}$ also show bonding with the Cu^+ ions along apex-equatorial boron edges.[10]

Presumably cage-opening occurs *via* the formation of bridge hydrogens between apex and equatorial borons. Bond rupture and coordination of ethyl sulfide between the equatorial borons result with the ethyl sulfide coordinated borons now becoming the 6 and 9 positions of the resulting $B_{10}H_{12}(Et_2S)_2$ structure. A plausible mechanism is depicted in eq 2 (the numbering system used is that for decaborane and only the "mouth" borons are numbered). The rupture of the cage bonds may involve a multicenter reaction with the Et_2SH^+ ion or simple displacement by some solvent ethyl sulfide.

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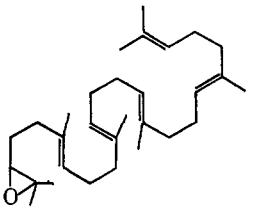
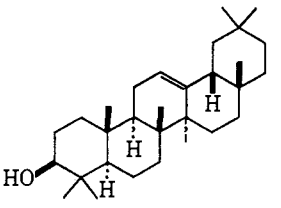
Enzymic Synthesis of β-Amyrin from 2,3-Oxidosqualene

Sir:

Recent studies have demonstrated that lanosterol is synthesized in the mammalian liver from 2,3-oxidosqualene (**1**)[1-3] under the influence of an enzyme, 2,3-oxidosqualene–sterol cyclase, which can be obtained from liver microsomes in a partially purified water-soluble form.[4] The separation of the squalene-to-sterol conversion into discrete oxidation and cyclization steps suggests a similar possibility for the biosynthesis of pentacyclic triterpenes and, therefore, a powerful experimental approach for studying the details of the remarkable rearrangement processes which are supposed to lead from the lupanyl[5] system to the triterpenes of the oleanane (β-amyrin), ursane (α-amyrin),[6] friedelane,[7] and other series. Since it has been shown that β-amyrin (**2**) is formed enzymically from squalene in a homogenate from germinating peas (*Pisum sativum*),[8] this system was selected for initial study. We report here an investigation which demonstrates that 2,3-oxidosqualene is indeed a precursor of β-amyrin in *Pisum sativum* and that the cyclizing enzyme can be obtained in water-soluble form.



1



2

Anaerobic incubation for 24 hr at 37° of ^{14}C-labeled 2,3-oxidosqualene with a cell-free homogenate from *Pisum sativum* led to isolation of a product which showed upon thin layer chromatography on silica gel about 35% of the radioactivity in a band with an R_f equal to that of β-amyrin. That this material was in fact primarily β-amyrin was demonstrated by the following experiments. Recrystallization (*ca.* 2 mμ-moles, 6000 counts/min) from ethanol–water with carrier β-amyrin (50 mg) led to a constant specific activity after the first crystallization (131, 106, 105, 104, 108 counts/min per mg). The combined fractions from this recrystallization experiment were acetylated with acetic anhydride in pyridine and chromatographed. The radioactivity was found in the zone characteristic of β-amyrin acetate using a thin layer of silica gel and 5% ethyl acetate–benzene for development. In addition, thin layer chromatography of acetylated biosynthetic material on a 20% silver nitrate–silica gel plate (3:2, chloroform–petroleum ether) showed the absence of labeled lanosterol and an R_f for the radioactive product identical with that of β-amyrin acetate. Vapor phase chromatography of acetylated labeled biosynthetic product using a glass column packed with 2% Epon 1001 on Diatoport S at 235° capable of separating α- and β-amyrin acetates showed >95% of the radioactivity in the β-amyrin acetate peak.

The intermediacy of squalene 2,3-epoxide in the biogenesis of β-amyrin is further supported by an experiment using 2,3-iminosqualene, which has been shown to be a potent inhibitor of enzymic cyclization of 2,3-oxidosqualene to lanosterol.[9] Incubation of ^{14}C-labeled squalene in the presence of 2,3-iminosqualene with a cell-free homogenate from *Pisum sativum* capable of converting squalene to β-amyrin[8] led to isolation by chromatography of about 2% of the radioactivity in a band of R_f equal to that of 2,3-oxidosqualene (using silica gel with 3% ethyl acetate–benzene as solvent). Dilution of this material with carrier 2,3-oxidosqualene and treatment with perchloric acid in aqueous glyme led to a product which upon chromatographic separation (using silica gel with 20% ethyl acetate–benzene as solvent) manifested radioactivity almost totally in the band of R_f corresponding to 2,3-dihydroxylated squalene.[2]

The solubilization and partial purification of this 2,3-oxidosqualene–β-amyrin cyclase from *Pisum sativum* has been effected by a procedure similar to that used in the purification of the 2,3-oxidosqualene–lanosterol cyclase from hog liver.[4] The cell-free homogenate[8] in pH 7.4 phosphate buffer (without added sucrose or glutathione) was treated at 0° with just sufficient sodium desoxycholate solution to effect clarification,

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and then an equivalent amount of calcium chloride solution was added to precipitate desoxycholate. The supernatant solution was separated after centrifugation, and the enzyme was precipitated from 30% ammonium sulfate solution. The precipitate obtained upon centrifugation (37,000*g*, 20 min) was taken up in cold phosphate buffer (pH 7.4) and centrifuged at 100,000*g* for 3 hr. The clear supernatant liquid was removed by pipet and assayed for total protein and activity in the anaerobic 2,3-oxidosqualene–β-amyrin conversion. The particle-free solution had a specific activity in cyclization to β-amyrin (per mg of protein) approximately 12 times that of the original microsomes.

Studies are continuing on the details of the enzymic formation of β-amyrin and other triterpenes.

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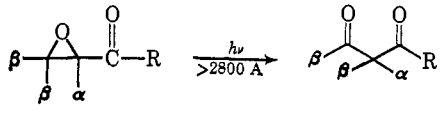
Photochemical Rearrangement of α,β-Epoxy Ketones. An Elaboration of the Mechanism

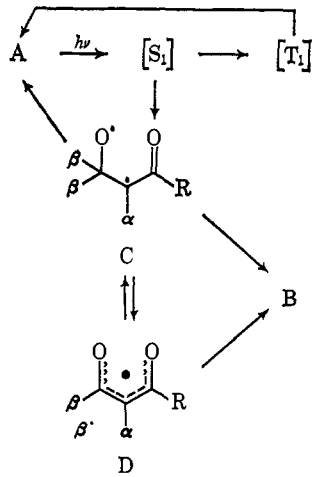
Sir:

The photorearrangement of α,β-epoxy ketones to β-diketones (eq 1) is characterized by an unusual order for the migrational aptitudes of various β groups (*e.g.*, $RCH_2 \gg C_6H_5$).[1,2] Suggestions concerning the



(1)

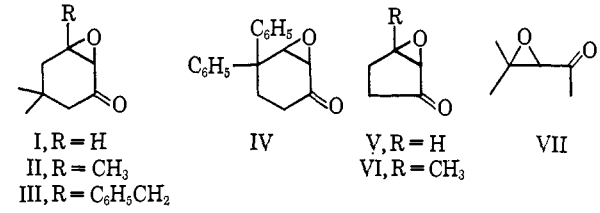
A B



(2)

mechanism of this transformation have been advanced,[1-4] and our recent work in this area has disclosed certain features of the rearrangement that support the elaboration of these views presented in eq 2.

The formation of intermediate C from a singlet [S_1] or triplet [T_1] excited state is theoretically reasonable,[5] and the reactions leading to this species constitute the true "photochemistry" of these compounds. Inasmuch as the epoxy ketone rearrangements are not sensitive to the presence of oxygen or changes in the solvent, and since the addition of known triplet quenchers (piperylene and 2,5-dimethyl-2,4-hexadiene were used in concentrations ranging from 0.1 to 9.0 *M*) did not affect the rate or course of rearrangement (specifically shown for II, III, VII, and VIII), *intermediate C appears to be formed predominantly from the initially produced* (S_1) *state.* Furthermore, acetophenone (0.5 *M*) did not function as a sensitizer for the rearrangement of II (0.3 *M*) in acetonitrile solution. The low quantum yield observed for some of these rearrangements (*e.g.*, *ca.* 0.03 for the conversion of VII to 3-methylpentane-2,4-dione) may indicate poor efficiency for the [S_1] → C transformation, or an unfavorable competition between rearrangement and oxirane ring formation from C. A preliminary study involving reduction of [T_1] by tri-*n*-butylstannane suggests that in the case of VII both factors are important.[6]

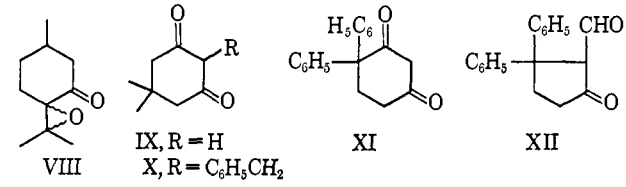


I, R = H
II, R = CH_3
III, R = $C_6H_5CH_2$

IV

V, R = H
VI, R = CH_3

VII

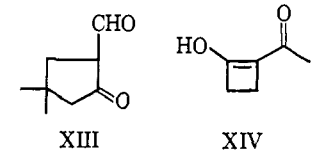


VIII

IX, R = H
X, R = $C_6H_5CH_2$

XI

XII



XIII XIV

A recent report[7] concerning the thermal decomposition of β-methyl-β-phenyl-β-peroxypropiolactone noted a fivefold preference for methyl migration *vs.* phenyl and suggested a 1,3-diradical intermediate similar to C. The implication that such a species can be generated by nonphotochemical pathways is supported by the isomerization and rearrangement of pulegone oxide diastereoisomers (VIII) at 200°.[1] A rationalization of the abnormal migrational aptitudes found in rearrangements proceeding from intermediate C is achieved by assuming that the migrating group must have radical characteristics. This feature can be incorporated in a fragmentation (two-step) mechanism involving the caged radical pair D, or in a single step path having a transition state resembling D in certain respects.[8]

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