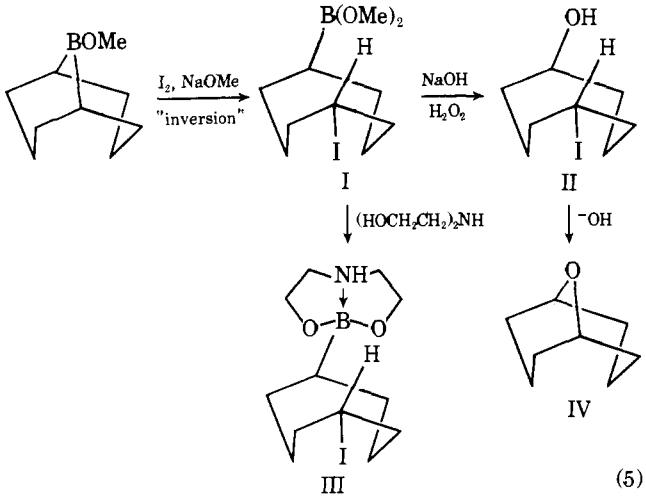
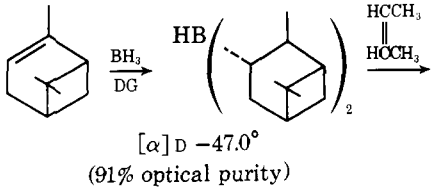
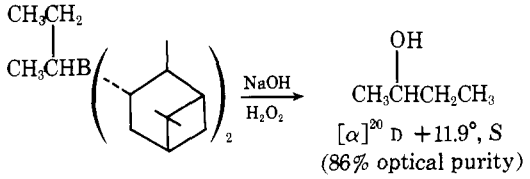


(5)

volve reaction of iodine at a bicyclic center. It was important to establish whether inversion would occur at a secondary center not involving this special structural feature. We selected diisopinocampheyl-2-butylborane[15] for study (eq 6). Note that the 2-butanol produced from (−)-α-pinene possesses the *S* configuration.

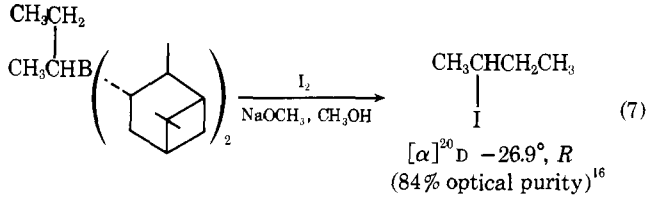


$[\alpha]_D$ −47.0°
(91% optical purity)



(6)

Treatment of the borane with iodine in the presence of sodium methoxide–methanol yields 2-iodobutane (*R*) with $[\alpha]^{20}D$ −26.9° (84% optical purity) (eq 7).



(7)

α-Pinene ($[\alpha]_D$ −47.1°) was converted to diisopinocampheylborane in diglyme and the latter treated with *cis*-2-butene as previously described.[15,9] The product, 0.200 mol, was divided into two equal parts. One-half was oxidized with alkaline hydrogen peroxide, yielding 2-butanol with $[\alpha]^{20}D$ +11.9°, whereas the second was treated with iodine and sodium methoxide–methanol (2 h). A 49% yield of 2-iodobutane was obtained, $[\alpha]^{20}D$ −26.9°. The alcohol possesses the *S* configuration, whereas the iodide possesses the *R*.

Consequently, it is evident that the reaction of organoboranes with iodine, induced by sodium methoxide, proceeds generally with inversion of the carbon–boron bond. This development not only provides a new synthetic route to *endo*-norbornyl and similar bicyclic iodides, but it makes available a promising new route to optically active iodides.

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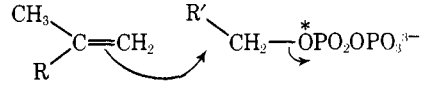
Received November 21, 1975

Application of Unreactive Analogs of Terpenoid Pyrophosphates to Studies of Multistep Biosynthesis. Demonstration That "Presqualene Pyrophosphate" Is an Essential Intermediate on the Path to Squalene

Sir:

Pyrophosphate monoesters play a dominating role in the biosynthesis of terpenoids, especially with reference to chain extension and ring formation.[1] The head-to-tail joining of isoprene units by carbon coupling, for example, involves intermolecular nucleophilic attack by a carbon–carbon double bond at a saturated carbon with displacement of a pyrophosphate leaving group:



Analogues of pyrophosphates in which the carbinol oxygen (O*, above) is replaced by methylene can reasonably be expected both to resist such enzymic C–C coupling and to function as selective enzyme inhibitors ("substrate analogue" type). In this communication we describe the synthesis of a series of these pyrophosphate analogues (C-substituted methylphosphonophosphates), the demonstration that they do inhibit biosynthetic processes involving pyrophosphate substrates as postulated, and an illustration of how this inhibition can be utilized to gain new information regarding multistep biosynthetic pathways.

Geranylmethylphosphonophosphate trilithium salt (**4**, R = geranyl) was synthesized starting with the reaction of geranyl bromide (**1**, R = geranyl) with 1 equiv of dimethyl lithiomethylphosphonate[2] in tetrahydrofuran (THF) at −78° to form phosphonic diester **2**[3] (60–70%). Cleavage of

Table I. Inhibition of Squalene Biosynthesis from Mevalonate (S_{10} Liver Preparation)

% inhibition	I/MEVAL[a] ratio × 10^3				
	I_{DMA}	I_{IPT}	I_{GER}	I_{FAR}	I_{PSQ}
20	2.5	1	0.25	0.25	6
50	45	33	1	1.25	22
80	262	170	4	4.8	150
90	375	325	12	35	250

[a] Concn of MEVAL = 2 mM in all experiments.

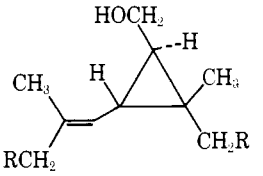
2 to diacid **3** was accomplished (ca. 80% yield) in two steps (base hydrolysis to monoester and subsequent demethylation with sodium iodide in dry methyl ethyl ketone at reflux[4]). Phosphorylation of **3** was effected via the phospho-

$$RBr + \underset{\mathbf{1}}{LiCH_2PO(OCH_3)_2} \longrightarrow \underset{\mathbf{2}}{RCH_2PO(OCH_3)_2} \longrightarrow$$

$$\underset{\mathbf{3}}{RCH_2PO(OH)_2} \longrightarrow \underset{\mathbf{4}}{RCH_2PO_2OPO_3^{3-}3Li^+}$$

R = geranyl

nomorpholidate,[5] and the resulting phosphonophosphate was isolated and purified chromatographically[5,6] as the trilithium salt **4** (ca. 50% yield). In a similar way three other isoprenoid phosphonophosphates of structure $RCH_2PO_2O\text{-}PO_3^{3-}$ $3Li^+$ were prepared: **5**, R = farnesyl; **6**, R = γ,γ-dimethylallyl; and **7**, R = isopentenyl. Finally, "presqualene alcohol" (**8**)[7,8] was converted to the phosphonophosphate **9** by a sequence starting with synthetic presqualene alcohol[8] (RCH_2OH) involving: (1) $RCH_2OH \rightarrow RCHO$ (Collins oxidation at 25° for 1 h in CH_2Cl_2, 88%); (2) $RCHO \rightarrow RCH{=}CHPO(OCH_3)_2$ (2 equiv of $Bu_3P{=}CHPO(OCH_3)_2$[9] in 1:1 THF-*n*-butyl alcohol for 3 h at 25°, 89%); (3) diimide reduction to $RCH_2CH_2PO(OCH_3)_2$ (excess diimide periodate, 85%); and (4) two-stage hydrolysis (82%) and phosphorylation to **9** (50%) as described above.



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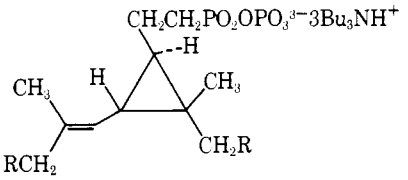

9

R = geranyl

These phosphonophosphates are referred to herein mnemonically according to the group attached to methylphosphonyl carbon; i.e., I_{GER}, I_{FAR}, I_{DMA}, I_{IPT} and I_{PSQ} correspond to **4, 5, 6, 7** and **9**, respectively. Thus I_{GER} is the analog of geranyl pyrophosphate.

The inhibition of squalene biosynthesis by the various phosphonophosphates was studied using C(5)-^{3}H and C(2)-^{14}C labeled mevalonate (MEVAL) with the rat liver S_{10} squalene synthetase preparation[10] (in 0.1 M Tris·HCl buffer of pH 7.5). Parallel anaerobic incubations were performed[11] with and without added phosphonophosphate inhibitor using an amount of enzyme sufficient to cause 1.0% conversion of 2 μmol of mevalonate to squalene after 10 min at 37° in the absence of inhibitor. The squalene produced (no inhibitor) increased linearly with time up to at least 20 min of incubation. Percent inhibition of squalene biosynthesis in the presence of inhibitor was measured for various ratios of inhibitor to mevalonate with the results summarized in Table I. Degree of inhibition as a function of inhibitor–mevalonate ratio is shown for the specific case of I_{GER} in Figure 1; similar curves were observed with the other phosphonophosphates. The observed effectiveness of inhibition follows the order $I_{FAR} \simeq I_{GER} > I_{PSQ} > I_{DMA} \simeq I_{IPT}$. In each case *complete* inhibition of squalene biosynthesis could be effected at an appropriate ratio of I/MEVAL.

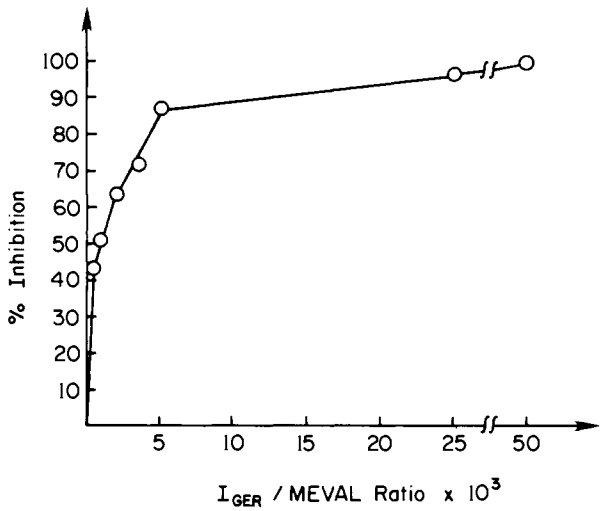

Figure 1. Inhibition of squalene biosynthesis from mevalonate (MEVAL) by I_{GER} (**4**) with the S_{10} rat liver squalene synthetase preparation. [MEVAL] = 2 mM.

The inhibition of kaurene biosynthesis from mevalonate in the cell-free kaurene synthetase from *Ricinus communis*[12] was studied with the inhibitors I_{FAR}, I_{GER}, I_{DMA}, and I_{IPT} with similar results. Again, stronger inhibition was observed for I_{FAR} or I_{GER} than for I_{DMA} or I_{IPT}, but kaurene biosynthesis could be completely blocked by any of the four inhibitors.

In both squalene and kaurene synthetase systems only extremely weak inhibition was observed by the phosphonates corresponding to the phosphonophosphates **4–7.** Further, perhydro I_{GER} (synthesized by hydrogenation of I_{GER} over Rh/C catalyst) was a very poor inhibitor of squalene or kaurene biosynthesis.

The fact that the biosynthesis of squalene from mevalonate by the S_{10} preparation can be completely inhibited by I_{PSQ} (at I_{PSQ}/MEVAL ≥ ca. 0.5) provides a strong indication that there is no other pathway than that via presqualene pyrophosphate. Because of the complexity of the presqualene pyrophosphate route from farnesyl pyrophosphate to squalene as compared with mechanistically similar but more direct routes, and because the formation of presqualene pyrophosphate is observed in the *absence* of NADPH, a cofactor for squalene biosynthesis, there has been some question as to whether presqualene pyrophosphate is essential to squalene biosynthesis or is off the major biosynthetic pathway (but still convertible to squalene).[13] It seemed to us that this matter could be resolved by further experiments using I_{PSQ}.

First it was established by experiment that the conversion of a *mixture* of C(2)-^{14}C labeled mevalonate and C(1)-^{3}H labeled presqualene pyrophosphate to ^{14}C or ^{3}H labeled

squalene by the S_{10} preparation could be *completely* inhibited by I_{PSQ}. In contrast, there was no inhibition of squalene biosynthesis from presqualene pyrophosphate at comparable I/MEVAL ratios by I_{GER}, I_{FAR}, I_{DMA}, or I_{IPT}, evidence that effective inhibition requires a close correspondence of substrate and inhibitor carbon structure. The same results were obtained with the microsomal liver preparation[14] (referred to herein as MLP) which effects squalene biosynthesis from farnesyl or presqualene pyrophosphates but not from C_5 or C_{10} precursors, both with regard to inhibition of squalene biosynthesis from presqualene pyrophosphate by I_{PSQ} and lack of inhibition by the other phosphonophosphates.

Incubation of 50 nmol of tritiated mevalonate, 25 nmol of unlabeled presqualene pyrophosphate, and 500 nmol of I_{PSQ} with sufficient S_{10} enzyme[15] to convert 12% of the mevalonate to squalene in the absence of I_{PSQ} yielded *no* tritiated squalene but showed a 9% conversion (*75% of expected maximum*) of mevalonate to tritiated presqualene pyrophosphate. For identification the labeled presqualene pyrophosphate was purified by thin layer chromatography (silica gel, *n*-propyl alcohol–11 N ammonium hydroxide 1.5:1, R_f identical with that of unlabeled presqualene pyrophosphate) and reincubated separately with both S_{10} enzyme and MLP enzyme to afford in each case tritium labeled squalene. Labeled squalene was identified unambiguously by chromatographic data and also by conversion to the crystalline thiourea complex which could be recrystallized to constant specific radioactivity. Further, characterization of the tritiated presqualene pyrophosphate produced in the above experiment was obtained by reduction with lithium aluminum hydride to labeled presqualene alcohol, chromatographically identical with authentic material (R_f 0.27 on silica gel plates using 2:1 pentane–ether for development). These experimental data indicate that I_{PSQ} can completely turn off squalene biosynthesis from mevalonate or presqualene pyrophosphate and also that presqualene pyrophosphate is formed and accumulated *under normal conditions of squalene biosynthesis from mevalonate* if I_{PSQ} is present. Given these facts and the specific inhibition of the presqualene pyrophosphate to squalene conversion by only I_{PSQ}, there seems to be no way to avoid the conclusion that presqualene pyrophosphate is an *essential intermediate* in squalene biosynthesis in liver; that is, there is no pathway from mevalonate to squalene which does not go through this intermediate.[16]

It seems apparent that the study of phosphonophosphate analogs can be helpful in the elucidation of biosynthetic pathways to terpenoids.[17]

References and Notes

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(10) G. Popják, *Methods Enzymol.*, 15, 438 (1969).
(11) See T. T. Tchen, *Methods Enzymol.*, 6, 509 (1963), for method of incubation. No dispersant (e.g., Tween 80) was used; inhibitor (or substrate in the case of presqualene pyrophosphate) was deposited as a film in the incubator tube by evaporation from benzene solution and mixed with the enzyme solution by agitation using a vortex mixer. Labeled squalene was purified by preparative thin layer chromatography on a silica gel plate (0.25 mm thickness of layer, 15 cm length) using 2% ether–98% petroleum ether for development (R_f 0.60 for squalene).
(12) C. A. West, *Methods Enzymol.*, 15, 481 (1969).
(13) See, for example, J. W. Cornforth, *Chem. Soc. Rev.*, 2, 1 (1973); I. Schechter and K. Bloch, *J. Biol. Chem.*, 246, 7690 (1971).
(14) See ref 10, pp 450–453.
(15) In this and all other experiments with the S_{10} system, NADPH, Mg^{2+}, and all other necessary cofactors had been added in the usual amounts.
(16) For other recent papers relevant to the role of presqualene pyrophosphate in the biosynthesis of squalene, see (a) F. Musco, J. P. Carlson, L. Kuehl, and H. C. Rilling, *J. Biol. Chem.*, 249, 3746 (1974); (b) G. Popják, H. Ngan, and W. Agnew, *Bioorg. Chem.*, 4, 279 (1975).

(17) This research was assisted financially by the National Science Foundation and the National Institutes of Health. We thank Professor Konrad Bloch and the members of his research group for numerous helpful discussions.

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Received November 24, 1975

Effect of Photoselection on Fluorescence-Detected Circular Dichroism

Sir:

In a recent study Turner et al.[1] have proposed that the circular dichroism, CD, of a fluorescent chromophore can be measured by detecting its fluorescence upon excitation by right-handed and left-handed circularly polarized light. The underlying assumption is that the excitation spectrum of a fluorescent chromophore parallels its absorption spectrum, i.e., that the measured fluorescence intensity of the chromophore depends exclusively on the amount of light absorbed by it. It was pointed out that such studies may be advantageous for the specific measurement of the CD of the fluorescent chromophores in biopolymers, thus eliminating contributions from nonfluorescent chromophores with overlapping absorption bands, which are often also present in the macromolecules.[1]

While the proposed method for measuring CD via emitted fluorescence intensity is promising and of much interest, it may be in serious error when applied to chromophores when rotatory Brownian motion is frozen (or restricted) during the lifetime of the excited state of the chromophore. This restriction may apply, for example, to a variety of native chromophores in biopolymers. The physical reason behind the complication which arises in frozen systems is as follows. The light absorbed by the system under study does not excite equally molecules of different orientations, since the probability of light absorption by a specific molecule depends on the orientations of its electric and magnetic dipole as well as electric quadrupole transition moments relative to the vector potential and direction of propagation of the light wave.[2a] In the case of circularly polarized light, the probability of excitation of a specific molecule thus depends on the sense of polarization. If rotatory Brownian motion does not randomize molecular orientations before light emission, different anisotropic populations of excited molecules contribute to the fluorescence upon excitation with right-handed or left-handed circularly polarized light. The observed intensity of fluorescence depends not only on the number of excited molecules, but also on the distribution in space of