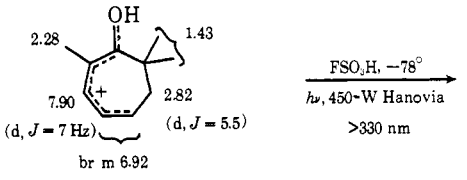
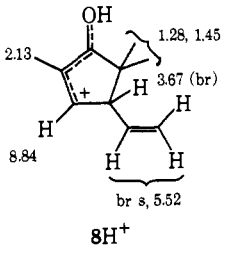
presence of a tertiary α carbon atom. The same products were formed about 10 times faster when the solvent was trifluoroethanol (75% conversion of **6** to **7** and **8** in 2 hr).

A vinyl cyclopentenone such as **8** has not been observed previously in any cycloheptadienone photoisomerization. Since polar solvents favor its formation, **8** is believed to arise from a π–π^* state of the cycloheptadienone.[5] To test this possibility, **6** was irradiated in FSO_3H at −78°. The sole initial photoproduct was $8H^+$,[6] which can only arise from a π–π^*



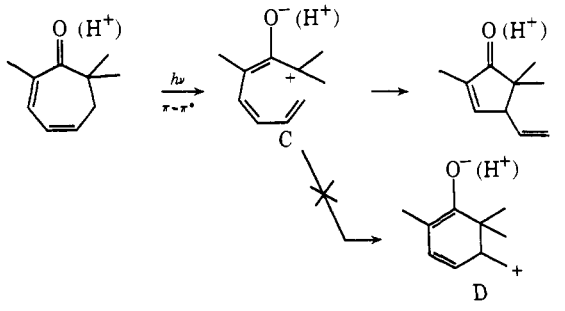
$6H^+$ [$\lambda_{max}^{H_2SO_4}$ 399 nm (ε 4730)]



$8H^+$

state of the protonated cycloheptadienone. This results is in striking contrast with the irradiation of protonated eucarvone.[1f]

The results can be rationalized using an intermediate such as C (analogous to A). In contrast with A, C will have the greatest positive charge density at the other end of the unsaturated system because of the different location of the *gem*-dimethyl group. Cyclization to D



(analog of A → B) is highly unfavorable, whereas cyclopentenone formation encounters no difficulties.[7]

One may generalize these results and propose that in reactions from the π–π^* state of 2,4-cycloheptadienones (or their protonated forms), substituents which stabilize a positive charge will favor products of the type **3** if located at C-6, and of the type **8** if located at C-7. In preliminary confirmation of this proposal, we have found that a single methyl substituent added to C-7 of eucarvone (*i.e.*, 2,6,6,7-tetramethyl-2,4-cycloheptadienone) is sufficient to divert some of the π–π^* product to the cyclopentenone type.[8]

It is clear that the presence or absence of particular substituents at various ring positions can have a profound effect on the structure of cycloheptadienone photoproducts, and that eucarvone should not be considered a universal model for such systems.[9]

Acknowledgment. We are indebted to the National Institutes of Health (GM 15997) for their generous financial support.

(5) Preliminary experiments indicate that **7a** arises from an n–π^* state.

(6) The internal reference for the nmr spectra was $(CH_3)_4N^+BF_4^-$, δ 3.10. No thermal isomerization of $6H^+$ occurred at −78°. Prolonged irradiations give some $9cH^+$, $9tH^+$ and an unidentified ion, but these were shown to arise from the slow thermal rearrangement of $8H^+$ in FSO_3H.

(7) Analogous cyclizations of heptatrienyl cations to cyclopentenyl cations are well known (for examples, see T. S. Sorensen in "Carbonium Ions," Vol. 2, G. A. Olah and P. von R. Schleyer, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 807–835). Cyclization occurs in the most favorable sense, which in the present instance is to give a carbonium ion which is stabilized by the oxygen function.

(8) The addition of a single methyl at C-7 leads to stereoisomeric products; the chemistry of this system will be discussed in a separate paper.

(9) After this paper was submitted, a pertinent related paper appeared on the photoisomerization of 2,4-cycloheptadienone and its 2-methyl derivative [K. E. Hine and R. F. Childs, *J. Chem. Soc. D*, 145 (1972)]. D. I. Schuster and M. A. Tainsky have also studied the unsubstituted dienone (private communication from Professor Schuster). Both groups have independently recognized the atypicality of eucarvone, and the significant role which alkyl substitution can play in the photoisomerization of conjugated cycloheptadienones.

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Received January 15, 1972

Prephytoene Pyrophosphate. A New Intermediate in the Biosynthesis of Carotenoids

Sir:

The early steps of the biosynthesis of sterols and carotenoids proceed along nearly identical pathways of head-to-tail terpene condensation, which are followed by the head-to-head condensation of two polyprenyl pyrophosphate moieties. The sterol precursor squalene is the product of a head-to-head condensation of farnesyl pyrophosphate, while phytoene, the first 40-carbon carotenoid, arises from a similar condensation of geranylgeranyl pyrophosphate.[1] Recently, we isolated, characterized, and unambiguously synthesized presqualene pyrophosphate, an intermediate between farnesyl pyrophosphate and squalene.[2–4] Since these two pathways are analogous, a 40-carbon cyclopropylcarbinyl pyrophosphate ester would be anticipated as precursor to phytoene. We now report the isolation, characterization, and synthesis of this precursor **1a**, for which we propose the name prephytoene pyrophosphate.

Geranylgeraniol was prepared by a coupling[5] of **2** and *trans*-**3** in tetrahydrofuran[6] followed by reduction of the initial coupling product with lithium in ethylamine at −78°. The geranylgeraniol so obtained (87% all trans) was further purified by successive recrystallizations of the diphenylurethane derivative,[7]

(1) (a) T. W. Goodwin, *Biochem. J.*, **123**, 293 (1971); (b) T. C. Lee and C. O. Chichester, *Phytochemistry*, **8**, 603 (1969).

(2) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **245**, 4597 (1970).

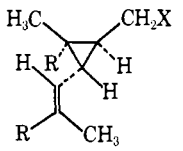
(3) H. C. Rilling and W. W. Epstein, *J. Amer. Chem. Soc.*, **91**, 1041 (1969).

(4) L. J. Altman, R. C. Kowerski, and H. C. Rilling, *ibid.*, **93**, 1782 (1971).

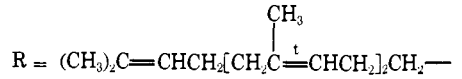
(5) J. F. Biellmann and J. B. Ducep, *Tetrahedron Lett.*, 3707 (1969).

(6) E. E. van Tamelen, P. McCurry, and U. Huber, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1294 (1971), and references cited therein.

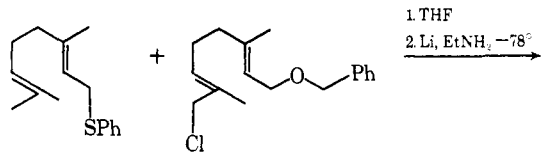
(7) R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, **28**, 1086 (1963).



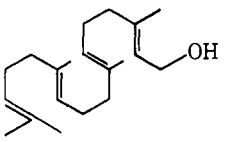
1a, X = OPP
1b, X = OH



which upon saponification yielded geranylgeraniol[8] (98% all trans). Geranylgeranial, prepared by oxidation of geranylgeraniol with MnO_2,[9] was reduced with $LiAl^3H_4$ to give the tritiated alcohol. A second



2 **3**



geranylgeraniol

sample was prepared by rearranging a commercial isomeric mixture of geranyllinalool to a mixture of geranylgeraniols.[10] The all-trans isomer was isolated by repeated column chromatography on silica gel impregnated with $AgNO_3$. Reduction of the corresponding methyl ester by $LiAl^3H_4$ gave the tritiated alcohol.

Synthetic **1b**[8] was prepared by the addition of the allylic diazo compound derived from geranylgeranial to a solution of geranylgeraniol and zinc iodide in ether[4,11] and purified by preparative layer chromatography (silica gel–HF, ethyl ether–carbon tetrachloride, 15:85). Reduction of the corresponding aldehyde with $LiAl^3H_4$ gave the tritiated alcohol.

The alcohols were phosphorylated chemically and the pyrophosphate esters isolated by chromatography on Dowex 1-X8 (formate form).[2]

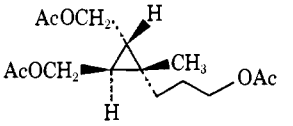
A species of *Mycobacteria* which produces carotenoids in response to light was the source of enzymes for these experiments.[12] These bacteria were photoinduced for 4 hr at 25° by shaking under 200 ft-candles of fluorescent light in 0.1 *M* phosphate buffer, pH 7.4, containing 1% glucose and 0.1% $(NH_4)_2SO_4$. After harvesting and washing, the cells were suspended in an equal volume of phosphate buffer and ruptured in a French pressure cell. After the addition of a few crystals of DNAse, the viscous extract was made 10^{-3} *M* in $MgCl_2$ and kept at 30° for 15 min. For incubations, the substrates were mixed with Tween 80 in either benzene or methanol. After removal of solvent, the bacterial extract was added. Incubations were for 3 hr at 30° under N_2.

On incubation of [^{3}H]geranylgeranyl pyrophosphate with the bacterial extract, a substantial amount of radioactivity was converted to hydrocarbons. After hydrogenation, analysis by glpc showed 25% of the radioactivity associated with lycopersane, while the majority of the radioactive material was of lower molecular weight. This experiment demonstrated the extract was capable of synthesizing carotenoids from geranylgeranyl pyrophosphate.

Thin-layer chromatography of butanol extracts from similar incubations showed incorporation of tritium from geranylgeranyl pyrophosphate into a region with an R_f corresponding to that of **1a**. For characterization of the biosynthetic prephytoene pyrophosphate, a larger amount was prepared and purified by ion-exchange chromatography.[2] The radioactive compound thus obtained cochromatographed with synthetic **1a** on buffered thin-layer plates (chloroform–methanol–water, 60:40:9, R_f 0.45). Both the synthetic and biosynthetic materials were converted to carotenoids by the bacterial extract in 34–45% of theoretical yield.

Treatment of the biosynthetic precursor with $LiAlH_4$ yielded an alcohol and a hydrocarbon fraction.[13] This alcohol cochromatographed by thin-layer chromatography with synthetic **1b** (benzene–ethyl acetate, 90:10, R_f 0.44), The acetate esters of these compounds also cochromatographed (benzene, R_f 0.32).

Biosynthetic alcohol **1b** was ozonized and acetylated to yield **4**, which cochromatographed with synthetic standard,[14] providing additional evidence for the cyclopropane ring in **1b**.



4-OAc

The evidence presented provides clear and definitive proof for the structure of prephytoene pyrophosphate and demonstrates that this compound is an intermediate between geranylgeranyl pyrophosphate and phytoene. The mechanism for the conversion of geranylgeranyl pyrophosphate to phytoene would be similar to those proposed for squalene synthesis from farnesyl pyrophosphate except for proton loss rather than hydride reduction for the final step.[4,14] The isolation of this cyclopropylcarbinyl pyrophosphate ester as the product of the head-to-head condensation of two molecules of geranylgeranyl pyrophosphate extends and generalizes the mechanism found for squalene synthesis to other head-to-head terpenoid condensations.

Acknowledgment. Acknowledgment is made to the Research Corporation, to E. I. du Pont de Nemours and Co., to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and

(8) In agreement with the molecular structures are (a) microanalyses correct to within 0.3% of theory, (b) nmr (including shift studies utilizing tris(dipivalomethanota)europium[4]), (c) ir, and (d) mass spectral data.

(9) E. J. Corey, N. W. Gilman, and B. E. Ganen, *J. Amer. Chem. Soc.*, **90**, 5616 (1968).

(10) L. Ruzicka and G. Firmenich, *Helv. Chim. Acta*, **22**, 392 (1939).

(11) As in the synthesis of presqualene alcohol, an epimeric product in approximately equal yields was also produced.

(12) H. C. Rilling, *Biochim. Biophys. Acta*, **79**, 464 (1964).

(13) A procedure that has been shown to reduce presqualene pyrophosphate to presqualene alcohol without rearrangement.[2]

(14) H. C. Rilling, C. D. Poulter, W. W. Epstein, and B. Larsen, *J. Amer. Chem. Soc.*, **93**, 1783 (1971).

to the National Institutes of Health (GM 08321) for partial support of this research.

(15) Alfred P. Sloan Research Fellow, 1971–1973.
(16) Research Career Development Awardee of the National Institutes of Health.

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Phenyl Esters for C-Terminal Protection in Peptide Synthesis

Sir:

The potential of phenyl esters as intermediates in peptide synthesis has been pointed out,[1] but the preliminary experiments have not been followed up hitherto. We were prompted to resuscitate the method by the need to synthesize peptides with the awkward sequence[2] -Asp-Gly- in the course of our lysozyme program. We now describe practical conditions for synthesis of oligopeptide phenyl esters, and for *removal of the phenyl group under very mild conditions without any racemization.*

A synthesis of the protected sequence 11–16 of calcitonin M exemplifies the construction of intermediates and the disposition of phenyl esters to crystallize. Treatment of Z-Phe-OPh[3] with HBr–HOAc yielded H_2^+-Phe-OPhBr$^-$, mp 232–233°,[4] which was coupled (mixed anhydride with isobutyl carbonate) with Z-Asp(OBu^t)-OH. The dipeptide derivative (68%) was an oil, which yielded, however, crystalline H_2^+-Asp(OBu^t)-Phe-OPhCl$^-$, mp 153–156° (78%), on hydrogenation (10% palladium/carbon) in dimethylformamide containing 1 equiv of HCl in dioxane.[5] The tripeptide derivative, Z-Gln-Asp(OBu^t)-Phe-OPh, mp 192–194°, was obtained (78%) by coupling (mixed anhydride with pivalic acid) with Z-Gln-OH, and then the tetrapeptide derivative, mp 137–139° (83%), by hydrogenolysis and coupling (mixed anhydride with isobutyl carbonate) with Z-Thr(Bu^t)-OH. Hydrogenolysis yielded the tetrapeptide phenyl ester, mp 119° (87%), which was coupled (dicyclohexylcarbodiimide–1-hydroxybenzotriazole)[6] with Z-Thr(Bu^t)-Tyr-OH. The resultant Z-Thr(Bu^t)-Tyr-Thr(Bu^t)-Gln-Asp(OBu^t)-Phe-OPh, mp 211–214° (58%), was hydrolyzed in 15 min at 20°, pH 10.5 (autotitrator), in aqueous dimethylformamide containing 0.80 equiv of hydrogen peroxide yielding the carboxylic acid, mp 193° dec (63%). In general, hydrolysis of C-terminal phenyl esters of peptide derivatives was accomplished efficiently in times between 7 and 20 min at pH 10.5, 20°, in mixtures of water (about 40%) and an organic solvent, which could be acetone, dioxane, or dimethylformamide.

The extraordinary susceptibility of phenyl esters to nucleophilic attack by peroxides is well documented by Jencks and Gilchrist.[7] Presumably the peptide peracids are the initial product, but conversion to the carboxylic acids is rapid, and only the latter have been isolated. Decomposition of peracids is a general phenomenon,[8] which is especially marked in alkaline solution in the case of the chloroacetyl compound,[9] which must have a pK_a similar to that of an α-amido acid; presumably the electron withdrawing of the α substituent promotes decomposition of the peracid. We have investigated racemization during peroxide-catalyzed hydrolysis by the general method of Manning and Moore,[10] which would easily detect 0.1% of the L,D dipeptide;[11] no trace was found in hydrolyses of the L,L dipeptide esters, Z-Ala-Phe-OPh and Z-Leu-Ala-OPh. In the absence of peroxide the rate of hydrolysis was an order of magnitude less and much racemization was observed. In contrast to the results with phenyl esters, normal saponification of the corresponding methyl esters (1 equiv of 0.25 *N* NaOH diluted with 3 vol of acetone, 1 hr at 20°) gave, respectively, 2.8 and 0.8% racemization. To our knowledge this danger of racemization in an *unactivated* peptide methyl ester has not been reported previously.

A check with Z-Asp(OBu^t)-Gly-OPh (*viz.* successive peroxide-catalyzed hydrolysis, hydrogenolysis, and treatment with trifluoroacetic acid) confirmed the absence of an $\alpha \rightarrow \beta$ aspartyl shift.[12] An obvious risk in peroxide catalysis is destruction of indole and sulfur side chains. Indeed BOC-Met-OPh yielded 78% of the sulfoxide and 9% of sulfone under the standard conditions, but oxidation was not detected when 30 mol equiv of dimethyl sulfide was added. Studies with BOC-Ala-Cys(Acm)-Gly-OPh and Z-Trp-Gly-OPh showed that destruction of the side chains was completely obviated by inclusion of dimethyl sulfide, which did not diminish the rates of hydrolysis.[13]

The heptapeptide derivative, Z-Asp(OBu^t)-Ile-Thr(Bu^t)-Ala-Ser(Bu^t)-Val-Gly-OPh, mp 260–262° dec, was synthesized stepwise from the C-terminus as in the first example above, except that 2,4,5-trichlorophenyl esters were used in adding the residues of Val, Ala, Ile, and Asp(OBu^t). All the intermediate *N*-carbobenzoxy phenyl esters were crystalline and their hydrolysis was tested in each case. The autotitrator showed reaction to be complete in 15 min (dipeptide), 7 min (tri-

(1) G. W. Kenner, *Angew. Chem.*, 71, 741 (1959).
(2) M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluscec, and O. Kócy, *Biochemistry*, 7, 4069 (1968).
(3) Abbreviations according to IUPAC-IUB tentative rules, *Biochemistry*, 5, 2485 (1966), and "Peptides 1969," E. Scoffone, Ed., North-Holland Publishing Co., Amsterdam, 1971, p xvii.
(4) All compounds gave satisfactory CHN (±0.4) and amino acid analyses (±5%). Cited yields are of analytically pure products.
(5) If methanol was included in the solvents for hydrogenolyses, some transesterification was detected.
(6) W. König and R. Geiger, *Chem. Ber.*, 103, 788 (1970).
(7) P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, 90, 2622 (1968).
(8) J. d'Ans and W. Frey, *Z. Anorg. Allg. Chem.*, 84, 145 (1913); D. Swern in "Organic Peroxides," Vol. 1, Wiley-Interscience, New York, N. Y., 1970, Chapter 6.
(9) E. Koubek, M. L. Haggett, C. J. Battaglia, K. M. Ibne-Rasa, H. Y. Pyun, and J. O. Edwards, *J. Amer. Chem. Soc.*, 85, 2263 (1963).
(10) J. Manning and S. Moore, *J. Biol. Chem.*, 243, 5591 (1968).
(11) JEOLCO JLC-5AH analyzer.
(12) Retention times of α and β dipeptides at 58 ml/hr, pH 3.25 buffer, were 88 and 42 min (column at 57°).
(13) In other examples, which do not have C-terminal Gly, it has later been found necessary to add intermittently fresh portions of hydrogen peroxide, usually no more than 3 equiv, in order to maintain the rate of hydrolysis. The products were, however, still of good quality.