Tetrahedron Letters No.32, pp. 3089-3094, 1967. Pergamon Press Ltd. Printed in Great Britain.

ACID CATALYZED HYDROLYSIS OF NERYL PYROPHOSPHATE AND GERANYL PYROPHOSPHATE.

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(Received 16 May 1967)

We have been interested for some time in the biosynthesis of monoterpenes in Pinus radiata, and we have shown the formation of some of the phosphorylated intermediates of cholesterol biosynthesis in the tissues of this species (1)(2). Several authors (3)(4) have postulated geranyl pyrophosphate as the nearest precursor of cyclic monoterpenes, assuming that the steps in this process would be the same as in the biosynthesis of cholesterol. The unlikeliness of a ring closure between C_1 and C_6 of geranyl pyrophosphate, due to its trans configuration at the allylic double bond has not been taken into account. It may be foreseeable, that the cis isomer, neryl pyrophosphate is more likely to undergo cyclization after loss of the pyrophosphate group, and may be thus a more probable precursor for the formation of cyclic monoterpenes. Zeitschel (5) inferred the cis configuration of nerol from the fact that it cyclizes easily upon prolonged acid treatment, whereas geraniol reacts much slower. Miller and Wood (6) have reported the formation of limonene from the diphenylphosphate ester of nerol and of myrcene and ocymene from the geranyl ester.

Elimination of pyrophosphate from neryl or from geranyl pyrophosphate may lead in both cases to the formation of the same carbonium ion $\frac{R}{CH_3}\!\!>\!C\!=\!\overset{+}{C}\!-\!\underset{H}{CH_2}$, which may stabilize either to an open chain or to a cyclic compound. Thus,

before attempting enzymic experiments with labeled neryl or geranyl pyrophosphate, it was thought advisable to obtain kinetic information on the products of acid hydrolysis of these pyrophosphates and to establish any differences in their behaviour. Similar experiments on the rate of isomerization of alcohols, in order to compare their rate constants with those of pyrophosphate hydrolysis, and to complete the data of Zeitschel were also performed.

Experimental: Pure geraniol and nerol (courtesy of Messrs. Boake, Roberts Inc., London) were pyrophosphorylated (7)(8). The exact concentration of allylic pyrophosphates in solutions, as well as the nature of the alcohol was determined by gas-liquid chromatography on a butanediol-succinate (140°) or 2% ethyleneglycol-adipate (80°) column after complete hydrolysis with alkaline phosphatase from E.coli (Worthington Labs. New Jersey) and extraction of the alcohol with pentane. The amount of alcohols was estimated by measurement of peak areas and comparison with known amounts of standards.

Isomerization of alcohols was carried out in 140 mM solutions of alcohols in a 1:1 (v/v) mixture of acetone and buffer or acid. After different lapses of time the reaction was stopped with NaOH and the alcohols were extracted with pentane for quantitative gas chromatographic analysis. Hydrolysis of pyrophosphates (20 mM) was studied under similar conditions, but without the addition of acetone. Identity of the reaction products was established by comparison of their retention volumes with authentic samples. The effluents were also collected and the NMR spectra of the products were compared with those of authentic samples. No orthophosphate formation (9) was detected during the hydrolysis of the pyrophosphates. First order constants were obtained for all experimental times assayed up to 80% of completion. The figures in the tables represent averages of the kinetic constants obtained over the whole experimental period.

Results and conclusions: Acid catalyzed isomerization of geraniol and nerol produces a mixture of alcohols. However, the rate of isomerization of nerol to the cyclic α terpineol is more than 18 times greater than the corresponding isomerization of geraniol. It should also be noted that the rate of transformation of geraniol into the open chain linalool is greater than the

analogous isomerization of nerol. (Table I). These results complete the data of Zeitschel, and are in good agreement with them.

TABLE I.

Rates of acid-catalyzed isomerization of nerol and geraniol in acetone/0.1 N HCl (1:1 v/v) at 37°C.

Alcohol	Product	$k.10^3(min^{-1})$	$^{a}k_c.10^3$ (liters mol^{-1} min^{-1})
Nerol	α-terpineol	0.18 ± 0.03	
	linalool	0.13 ± 0.005	3.3 ± 0.5
	geraniol	0.006[b]	
Geraniol	α-terpineol	< 0.01	
	linalool	0.29 ± 0.06	2.7 ± 0.2
	nerol	0.0058[b]	

[a] Catalytic constants for H^+ calculated from the rates at different values of (H^+).

[b] Extrapolated from the values of k_c.

The values of the rate constants for the hydrolysis of neryl and of geranyl pyrophosphates (Table II) also show that the cis isomer (neryl pyrophosphate) forms α-terpineol 50 times faster than the trans isomer (geranyl pyrophosphate).

TABLE II.

Rates of acid-catalyzed hydrolysis of neryl and geranyl pyrophosphates in 0.05 N HCl.

	Product	$k.10^3(min^{-1})$	$^{a}k_c$(liters $mol^{-1}min^{-1}$)
Ner-PP	α-terpineol	227 ± 10	
	linalool	70 ± 8	5.4 ± 0.6
	nerol	9.7 ± 0.9	
Ger-PP	α-terpineol	3.9 ± 0.3	
	linalool	209 ± 10	4.0 ± 0.7
	geraniol	26.3 ± 2	

[a] Catalytic constants for H^+ calculated from the rates at different values of (H^+).

As in the isomerization of alcohols, the trans pyrophosphate forms linalool somewhat faster than its cis isomer.

The rate of isomerization of alcohols is several orders of magnitudes slower than the hydrolysis of the corresponding pyrophosphates, at comparable concentrations of H^+, (Tables I and II).

For a given configuration the ratio of products is roughly the same for the isomerization of the alcohol and for hydrolysis of its pyrophosphate. (Table III).

TABLE III.

Ratio of products $\frac{\alpha\text{-terpineol}}{\text{linalool}}$ obtained from the hydrolysis of the pyrophosphates and from the isomerization of the free alcohols.

Substrate	Product ratio	Substrate	Product ratio
Neryl pyrophosphate	2.94[a]	Nerol	1.42[a]
Geranyl pyrophosphate	0.021[b]	Geraniol	0.043[b]

[a] Average from 9 values obtained at different experimental conditions.
[b] Average from 5 values obtained at different experimental conditions.

Both isomerization and hydrolysis rate are dependent of H^+ and of total acid concentration, as in general acid catalysis. The rates of hydrolysis decline sharply with increase of pH (Table IV), as reported for other allylic pyrophosphates (10).

The nature of the rearrangement products of isomerization of alcohols and of hydrolysis of their pyrophosphates, as well as the similarity of the ratio of products from one given geometrical isomer suggests that these reactions involve the formation of a carbonium ion (11) as the rate limiting first step.

The 50 fold difference in the rates of formation of α terpineol from neryl pyrophosphate as compared with geranyl pyrophosphate could be explained assuming that the intermediate mesomeric carbonium ion resulting from the elimination of pyrophosphate may retain the cis configuration to a high degree (11). Our observations lend some support to the working hypothesis that the neryl

TABLE IV.

Effect of pH on the rate constants for the hydrolysis of neryl and geranyl pyrophosphates.

Conditions	Substrate	$k.10^3(min^{-1})$			
		α-terpineol	linalool	nerol	geraniol
KCl-HCl buffer 0.375 M pH 1.75	Ner-PP	290 ± 10	105 ± 20	25 ± 3	——
	Ger-PP	7.1 ± 0.9	390 ± 20	——	51 ± 2
Glicine-HCl buffer 0.375 M pH 2.75	Ner-PP	20.1 ± 0.6	7.46 ± 0.2	1,85 ± 0.08	——
	Ger-PP	0.38 ± 0.01	19.5 ± 0.6	——	2.49 ± 0.6
Phtalate-HCl buffer 0.375 M pH 3.65	Ner-PP	3.69 ± 0.8	1.51 ± 0.4	0.18 ± 0.01	——
	Ger-PP	0.09 ± 0.01	3.36 ± 0.1	——	0.83 ± 0.2
Na-Acetate-Acetic Acid buffer 0.375 M pH. 4.1	Ner-PP	1.31 ± 0.1	0.45 ± 0.07	0.05 ± 0.001	——
	Ger-PP	0.027 ± 0.005	1.00 ± 0.1	——	0.16 ± 0.04
Na-Acetate-Acetic Acid buffer 0.375 M pH 4.7	Ner-PP	0.67 ± 0.2	0.23 ± 0.01	——	——
	Ger-PP	——	0.78 ± 0.01	——	——
Succinate-Tris buffer 0.375 M pH 6.0	Ner-PP	0.14 ± 0.01	0.04 ± 0.006	——	——
	Ger-PP	——	0.05 ± 0.01	——	——

pyrophosphate may be the more probable precursor of cyclic monoterpenes in biological systems. Since both isomers form both linalool as well as α-terpineol, the participation of geranyl pyrophosphate cannot be excluded a priori. The difference in rates observed in the acid catalyzed cyclization may, however, suggest that such difference could be expected in a biological system, without invocation of enzyme specificity. Preliminary experiments with an enzyme system from P. radiata seedlings show the presence of higher amounts of radioactivity from ^{14}C labeled mevalonic acid in nerol than in geraniol. Further enzymic experiments with labeled precursors are in progress.

Acknowledgements: This work was financially supported by Grants FG-Ch-101 from the U.S. Department of Agriculture and AM 10714-01 from the National Institutes of Health, while one of the authors participated in the Exchange Program University of Chile-University of California. The authors are very indebted to Mrs. M. Pieber-Perretta for the NMR identification of the reaction products.

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