The runs are summarized in Table III and a typical run is detailed in Table IV. All runs except the first (marked with an asterisk) were followed to 73% conversion or more; one run with cyclohexyl bromide was followed to 97.5% completion without change in rate constant and one with *cis*-4-*t*-butylcyclohexyl bromide was followed to 99.1% completion with the rate constant being steady up to 86.3%. All rate constants, $k_T$, but one were free of drift between 25 and 75% reactions, as were, in most cases, the $k_S/k_T$ ratios, except in two runs where the ampoules were not flushed with nitrogen and the ratio was completely erratic and too high, evidently due to oxidation difficulties. The one run less satisfactory than the rest is that for *trans*-4-*t*-butylcyclohexyl bromide which reacts extremely slowly and was followed for over a month. Because of shortage of substance, only one ratio $k_S/k_T$ could be obtained, but this one definitely evidenced elimination, as did the isolation experiments (see below). The over-all specific rate, $k_T$, showed a downward drift. The constant $k_T$ reported for *trans*-4-*t*-butylcyclohexyl bromide in Tables I and III was averaged over the first 30% of the reaction (24,000 minutes).

**Controls.**—In addition to the controls previously performed[8] the following points were checked: *Consumption of iodine by olefin formed:* A solution of two drops of 4-*t*-butylcyclohexene in 7.5 ml. glacial acetic acid (the amount used for quenching) turned yellow on the addition of one drop of *ca.* 0.05 *N* iodine solution. *Oxidation of thiophenol during filling of ampoules:* A solution for a kinetic run was prepared in the usual way except that no substrate was added. The base and iodine titers (*ca.* 0.1 and 0.05 *N* solutions, respectively) at the times indicated were: zero, 4.60 ml., 12.61 ml.; 1110 min., 4.45 ml., 12.51 ml; 2913 min., 4.50 ml., 12.53 ml.; 17,995 min., 4.52 ml., 12.55 ml. Evidently the fluctuations in iodine titer are no greater than those in base titer and not much greater than the limits of ordinary titration accuracy, indicating no appreciable oxidation of thiophenol during the filling of the ampoules. The same point is also emphasized by the lack of drift of the $k_S:k_T$ ratio in the kinetic runs; if there had been initial oxidation, this ratio should have drifted downward.

**Isolation Experiments.**—The isolation of 4-*t*-butylcyclohexene and *trans*-4-*t*-butylcyclohexyl phenyl thioether from *cis*-4-*t*-butyl-cyclohexyl bromide and of the same olefin and the epimeric *cis*-thio-ether from the corresponding *trans*-bromide have been described previously.[16] We have also isolated cyclohexene from the reaction of cyclohexyl bromide with sodium thiophenolate. The previous reaction of *trans*-4-*t*-butylcyclohexyl tosylate (IV) with thiophenolate was repeated on one-third the previous scale.[8] Since control experiments showed that 4-*t*-butylcyclohexene codistils with ethanol and is hard to recover from the distillate, the reaction mixture, after standing one month, was poured into dilute aqueous potassium hydroxide and extracted with pentane. The pentane solution was washed with aqueous potassium hydroxide, water, and then three times with 20% aqueous calcium chloride to remove ethanol. It was dried over calcium chloride, concentrated through a column and distilled. There was obtained 0.90 g. (20%) of 4-*t*-butylcyclohexene, b.p. 63° (27 mm.) (lit.[8] 55–57° (15 mm.)) and 3.4 g. (40%) of *cis*-4-*t*-butylcyclohexyl phenyl thioether, b.p. 165° (7 mm.) (lit.[8] 184–186° (13 mm.). Both products were identified by infrared spectra.[8] The thioether, this time, crystallized, m.p. 39.7–40.6° after two recrystallizations from 95% ethanol.

Reaction of 19.3 g. (0.1 mole) of 2-octyl bromide with excess sodium thiophenolate under conditions similar to the ones previously used for 2-octyl tosylate[8] (but extending the reaction time to one week) gave 13.5 g. (84%) of 2-octyl phenyl thioether, b.p. 157–159° (15 mm.) (lit.[8] 158.5–160° (15 mm.)) as the only product isolated. The infrared spectrum of this thioether was identical with that of material obtained from the tosylate.[8]

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NOTRE DAME, IND.

# COMMUNICATIONS TO THE EDITOR

## ISOPENTENOL PYROPHOSPHATE ISOMERASE

*Sir:*

A product of mevalonic acid metabolism recently has been isolated[1,2] to which the structure of $\Delta^3$-isopentenol pyrophosphate (IsPP) has been assigned tentatively.[3] Concurrent investigations with authentic IsPP have established it as well as farnesol pyrophosphate (FaPP) as intermediate compounds in the biosynthesis of squalene.[4] More recently, studies have been directed to the sequence leading from IsPP to FaPP. A preparation derived from baker's yeast has been found to catalyze the migration of the unsaturated bond of IsPP to yield $\gamma,\gamma$-dimethylallyl pyrophosphate (DmalPP).

Chemically prepared[4] IsPP-1-$C^{14}$ was incubated with the yeast enzyme. Although the product could be distinguished from IsPP by virtue of its extreme acid lability, paper electrophoresis under alkaline conditions failed to separate the product from IsPP. Studies with IsPP$^{32}$ indicated that pyrophosphate was released on acidification of the product. It was anticipated that acid cleavage would occur at the –C–O– linkage and that if the original product were DmalPP, it would to some extent undergo allylic rearrangement[5] to form dimethylvinylcarbinol in addition to dimethylallyl alcohol. Cleavage with phosphatase, which acts at the –O–P– linkage should yield only dimethylallyl alcohol. Products of both such hydrolyses were analyzed and the results were in accord with that expected from the cleavage of DmalPP (Table I).

Preliminary data indicate that DmalPP is incorporated into FaPP and that IsPP-isomerase is the only iodoacetamide-sensitive[4] step in the enzymatic sequence between mevalonic acid and squalene. A special role is suggested for the sulfhydryl group in the isomerization such as the

(1) F. Lynen, in Ciba Foundation Symposium on Biosynthesis of Terpenes and Sterols, J. and A. Churchill Ltd., London, in press.

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(4) F. Lynen, H. Eggerer, U. Henning and I. Kessel, *Angew. Chem.*, **70**, 738 (1958).

(5) R. H. DeWolfe and W. G. Young, *Chem. Revs.*, **56**, 753 (1956).

TABLE I

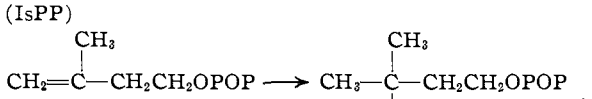
COMPOUNDS DERIVED FROM HYDROLYSATE OF PRODUCT

The fraction of yeast autolysate which was precipitated between 30% and 50% acetone concentration was dissolved and taken at *p*H 4.7 in the presence of added yeast ribonucleic acid.[6] The supernatant solution was taken to *p*H 5.5 with KOH and adsorbed on $Ca_3(PO_4)_2$ gel. The eluate obtained at *p*H 7 contained an isomerase activity of 1–3 μmole/mg. protein/hr. One mg. of enzyme was incubaetd in each of two identical tubes containing 20 μmoles of tris-(hydroxymethyl)-aminomethane buffer, *p*H 8, 6 μmoles of $MgCl_2$, 6 μmoles of $MgH_2$-ethylenediaminetetraacetate, and 0.4 μmole of IsPP-1-$C^{14}$ (3660 c.p.m.) in a total volume of 0.6 ml. After incubation for one hour at 37°, 0.06 ml. of 70% $HClO_4$ was added to tube 1, followed by sufficient KOH to bring the *p*H to 7. Next, 1.0 μmole each of dimethylallyl alcohol, dimethylvinylcarbinol and isopentenol were added. The mixture was extracted with ether and subjected to gas chromatography. Prostatic phosphatase was incubated with tube 2 which was then extracted and analyzed as was tube 1. The eluted alcohols were counted in a liquid scintillation counter.

| Substance | Elution time, minutes | Acid hydrolysis c.p.m. | Enzymatic hydrolysis c.p.m. |
|---|---|---|---|
| Dimethylvinylcarbinol | 8–12 | 1160 | 76 |
| Isopentenol | 24–30 | ... | ... |
| Dimethylallyl alcohol | 38–42 | 1125 | 3320 |

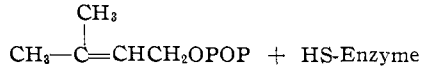
formation of an enzyme-substrate complex

(IsPP)



+ HS-Enzyme

(DmalPP)



Precedent for the sulfide catalyzed migration of an olefinic double bond is found in the Willgerodt reaction, in which a saturated intermediate state also has postulated.[7,8]

(6) O. Warburg and W. Christian, *Biochem. Z.*, **303**, 40 (1939).

(7) M. Carmack and D. L. F. DeTar, THIS JOURNAL, **68**, 2029 (1946).

(8) J. A. King and F. H. McMillan, *ibid.*, **68**, 632 (1946).

(9) National Institute of Neurological Diseases and Blindness, Bethesda, Maryland.

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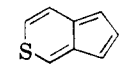
RECEIVED DECEMBER 27, 1958

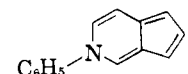
## SYNTHESIS OF CYCLOPENTA[c]THIAPYRAN AND 2-PHENYL-2-PYRIDINE[1]

*Sir:*

The recent report of substituted cyclopenta[b]-pyrans by Boyd[2] and the claims by Mayer[3] concerning cyclopenta[b]thiapyran prompt us to report the synthesis of cyclopenta[c]thiapyran (I) and 2-phenyl-2-pyrindine (II). Both of these molecules represent new heterocyclic structures which are iso-π-electronic with the monalternant aromatic hydrocarbon azulene.

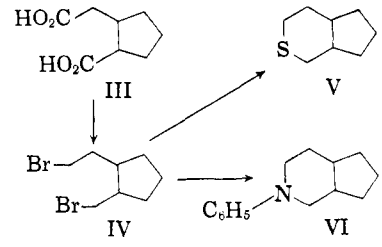


I



II

I and II were prepared as described. Esterification of cyclopropane-1-carboxy-2-acetic acid[4] (III) and reduction of the diethyl ester (or the diacid) with lithium aluminum hydride gave 80–85% of β-(2-hydroxymethylcyclopentanyl)-ethyl alcohol (b.p. 125–129° at 0.6 mm.) which was characterized as the diurethan (colorless plates, m.p. 105–107°, from carbon tetrachloride). Found for $C_{22}H_{26}N_2O_4$: C, 69.36; H, 6.67. Treatment of the diol with phosphorus and bromine afforded 63% of β-(2-bromomethylcyclopentanyl)-ethyl bromide (IV), b.p. 91–93° at 0.7 mm. Found for $C_8H_{14}Br_2$: C, 35.76; H, 5.24; Br, 58.71. Reaction of IV with sodium sulfide produced 64% of octahydrocyclopenta[c]thiapyran (V), b.p. 107–108° at 31 mm. Found for $C_8H_{14}S$: C, 67.74; H, 9.68; mol. wt., 142 (mass spectrograph). Vapor phase dehydrogenation of V over a Pd–C catalyst gave up to 32% of thrice recrystallized (from hexane) I as red plates m.p. 89–90.5°. Found for $C_8H_6S$: C, 71.89; H, 4.45; mol. wt., 134 (mass spectrograph). The ultraviolet and visible absorption spectra (hexane) resembled those of azulene and showed $\lambda_{max}$ in mμ (log ε) at 234 (4.15), 249 (4.28), 257 (4.28), 273 (4.34), 283 (4.52), 321 (3.45), 329 (3.38), 344 (3.11), 465 (3.08), 483 (3.04), 500 (2.97), 520 (2.54), 542 (2.54) and 565 (2.26). The basic character of I was shown by its solubility in 30% sulfuric acid and the change in absorption spectra in concentrated sulfuric acid to 208 (4.08), 240 (3.95), 293 (3.48) and 332 (4.18). The compound was degraded slowly by dilute sulfuric acid and glacial acetic acid and was decomposed by alumina. It was stable to alcoholic alkali and to sublimation at 70° and 60 mm.



Reaction of IV with aniline and sodium carbonate afforded 68% of 2-phenylperhydro-2-pyrindine (VI), b.p. 125–127° at 1.3 mm. Found for $C_{14}H_{19}N$: C, 83.51; H, 9.63; N, 7.27; mol. wt. of picrate, 430 (spectroscopic).[5] The structure of VI was shown by nitrosation and subsequent cleavage with base to form the known perhydro-2-pyrindine (m.p. of picrate, 142.5–143.5°).[6] Vapor phase dehydrogenation of VI over a Pd–C catalyst gave up to 25% of thrice recrystallized (fro

(1) Support for a part of this work by contracts DA-04-200-ORD 601 and DA-04-200-ORD 715 with the Office of Ordnance Research, U. S. Army, is gratefully acknowledged.

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