conditions may be useful practically and interesting theoretically.

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The Reaction of Vinyllithium with Tetraphenylphosphonium Bromide and the Formation of Phosphinemethylenes by RLi Addition to Vinylphosphonium Halides[1]

Sir:

The action of phenyllithium on tetraphenylphosphonium bromide results in formation of pentaphenylphosphorus,[2] but on the other hand, the reaction of methyl- or ethyllithium with tetraphenylphosphonium bromide produces benzene and the respective triphenylphosphinealkylidene as the major product.[3] Therefore it was of interest to us to determine which (if either) type of behavior would be observed in the reaction of vinyllithium with tetraphenylphosphonium bromide. In particular, we were interested in the possible stability of the unknown $(C_6H_5)_4PCH{=}CH_2$. Our study has shown that still a third type of behavior is possible in the $[(C_6H_5)_4P]Br$–RLi system.

When ethereal vinyllithium (25.4 mmoles, obtained *via* the procedure involving solid vinyllithium[4]) was added to a slurry of tetraphenylphosphonium bromide (25.7 mmoles) in 50 ml. of ether, a reddish brown solution was formed during rapid consumption of the organolithium reagent. Gas chromatographic analysis of the volatile products obtained in a high-vacuum bulb-to-bulb distillation and work-up of the residue showed that styrene[5] (65%) and triphenylphosphine[6] (82%) were the major products of the reaction. The main reaction, therefore, proceeded according to the following equation.

$$[(C_6H_5)_4P]Br + CH_2{=}CHLi \longrightarrow (C_6H_5)_3P + C_6H_5CH{=}CH_2 + LiBr$$

Two possible mechanisms have been considered for this reaction. First, the reaction possibly could involve initial formation of $(C_6H_5)_4PCH{=}CH_2$, followed by its subsequent decomposition to the observed products. Alternatively, an exchange mechanism was considered which could lead to the products by the following steps.

$$[(C_6H_5)_4P]Br + CH_2{=}CHLi \longrightarrow [(C_6H_5)_3PCH{=}CH_2]Br + C_6H_5Li \quad (1)$$

$$[(C_6H_5)_3PCH{=}CH_2]Br + C_6H_5Li \longrightarrow (C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5 + LiBr \quad (2)$$

$$(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5[7] \longrightarrow (C_6H_5)_3P + C_6H_5CH{=}CH_2 \quad (3)$$

Precedence exists for step 2; addition of nucleophiles to vinylphosphonium salts has been reported to occur readily.[8,9] However, decomposition (or rearrangement, followed by decomposition) of a phosphinemethylene such as $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5$ is not very likely. Nevertheless, this point was investigated by examining independently step 2 of the above sequence. In a typical experiment, a suspension of 16.0 mmoles of vinyltriphenylphosphonium bromide[10] in 95 ml. of ether was treated with 15.5 mmoles of phenyllithium in ether. The mixture became deep red and most of the solid disappeared. After several hours, acetone was added and the reaction mixture was heated at reflux for *ca.* 8–10 hr. All volatiles were distilled off under high vacuum. Gas chromatographic analysis showed the distillate to contain benzene[11] (44%), styrene (0.4%, but in other experiments yields of up to 5% were obtained), and 2-methyl-4-phenyl-2-butene[12] (in 33% yield, the product expected from the reaction of $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5$ with acetone). Small amounts of triphenylphosphine also were found. In an experiment in which the vinylphosphonium salt was added slowly in small portions to an excess of phenyllithium, very similar (59% benzene, 5% styrene, 32% 2-methyl-4-phenyl-2-butene) results were obtained. These experiments are of interest in several respects. They show that the sequence 1–3 does not occur in the vinyllithium–tetraphenylphosphonium bromide reaction, leaving a route *via* transient $(C_6H_5)_4PCH{=}CH_2$ as the best possibility. They indicate that phenyllithium attacks vinyltriphenylphosphonium bromide *at three different sites:* (a) to a minor extent at phosphorus, giving styrene and triphenylphosphine, presumably by way of $(C_6H_5)_4PCH{=}CH_2$; (b) to a major extent in a Michael-type addition to the activated double bond, leading to $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5$; and (c) at the vinyl group's α-C–H bond, which is rendered more acidic by the adjacent electron-withdrawing phosphonium function, to give benzene and $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}{=}CH_2$.[13] The latter should in principle react with acetone, producing 1,1-dimethylallene, but none could be found. Other attempts to isolate allenes when other carbonyl substrates were used also were unsuccessful.[14]

This procedure based on $[(C_6H_5)_3PCH{=}CH_2]Br$ may be of value in some cases where a particular phosphonium halide of structure $[(C_6H_5)_3PCH_2CH_2R]X$ is for some reason unavailable.[15] A more practical procedure goes directly from $[(C_6H_5)_3PCH_2CH_2Br]Br$[16] to $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5$ (again in *ca.* 35% yield) by reaction of the former with two molar equivalents of phenyllithium, the first equivalent being used to dehydrobrominate the β-bromoethylphosphonium salt *in situ*.

(1) Part VIII of the series "Studies in Phosphinemethylene Chemistry", Part VII: D. Seyferth and J. M. Burlitch, *J. Org. Chem.*, **28**, 2463 (1963).

(2) G. Wittig and M. Rieber, *Ann.*, **562**, 187 (1949).

(3) D. Seyferth, J. K. Heeren, and W. B. Hughes, *J. Am. Chem. Soc.*, **84**, 1764 (1962).

(4) D. Seyferth and M. A. Weiner, *ibid.*, **83**, 3583 (1961).

(5) Identified by its infrared spectrum and retention time and by conversion of a g.l.c. sample to $C_6H_5CHBrCH_2Br$, m.p. 71.5–73°.

(6) Identified by melting point and mixture melting point and by conversion of all the $(C_6H_5)_3P$ to known $[(C_6H_5)_3PCH_3]I$.

(7) Or a rearrangement product, such as $(C_6H_5)_3\overset{+}{P}{-}CH_2\overset{-}{C}HC_6H_5$.

(8) G. Wittig, H. Eggers, and P. Duffner, *Ann.*, **619**, 10 (1958).

(9) M. Grayson and P. T. Keough, Abstracts of Papers, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, p. 64Q.

(10) M.p. 185–187°. Prepared by dehydrobromination of $[(C_6H_5)_3PCH_2CH_2Br]Br$ with moist silver oxide.

(11) Identified by means of its infrared spectrum and retention time.

(12) N.m.r. and infrared spectra were consistent with this structure and identical with spectra of an authentic sample prepared by the reaction of triphenylphosphineisopropylidene and phenylacetaldehyde.

(13) It is to be noted that H. Gilman and R. A. Tomasi [*J. Org. Chem.*, **27**, 3647 (1962)] have reported the reaction of phenyllithium with $[(C_6H_5)_3PCH{=}C(C_6H_5)_2]Br$ to give $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}{=}C(C_6H_5)_2$.

(14) In a recent paper it has been reported that the Wittig reaction apparently is not applicable to the synthesis of allenes of types $R_2C{=}C{=}CHR$ and $R_2C{=}C{=}CH_2$ [G. Wittig and A. Haag, *Chem. Ber.*, **96**, 1535 (1963)]. Therefore our failure to isolate the expected terminal allenes is not surprising.

(15) The example given above is a case in point. We have found that pure $[(C_6H_5)_3PCH_2CH_2C_6H_5]Br$, required for the generation of $(C_6H_5)_3\overset{+}{P}{-}\overset{-}{C}HCH_2C_6H_5$ by standard methods, cannot be prepared readily by the reaction of β-bromoethylbenzene with triphenylphosphine because of complicating side reactions.

(16) M.p. 190.5–192.5° N.m.r. spectrum is consistent with this structure; prepared *via* $[(C_6H_5)_3PCH_2CH_2OH]Br + HBr$ (concd.).

The reaction of methyllithium with vinyltriphenylphosphonium bromide in ether–tetrahydrofuran medium can be understood in the same manner, although the products observed account for only *ca.* 30% of the lithium reagent consumed. When the reaction mixture was quenched with cyclohexanone, benzene (17%), *n*-propylidenecyclohexane (13%), and methylenecyclohexane (6%) were obtained.

All reactions were carried out under an atmosphere of prepurified nitrogen. All new compounds reported had satisfactory analyses. Our work in this area is continuing.

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(17) (a) Alfred P. Sloan Research Fellow; (b) National Science Foundation Graduate Fellow, 1960–1963; National Institutes of Health Predoctoral Fellow, 1963–1964; (c) Fellow of the M.I.T. School for Advanced Study, 1961–1962.

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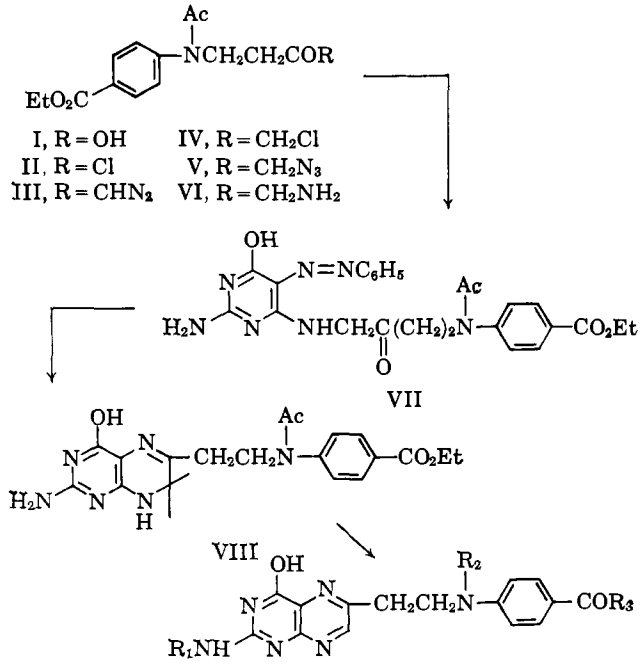
Tetrahydrohomofolate, a Specific Inhibitor of Thymidylate Synthetase

Sir:

This communication describes the synthesis and biological properties of homofolic acid (a trivial name for the compound possessing an additional methylene group between positions 9 and 10 of folic acid) and its reduced forms, dihydrohomofolate (homofolate-H_2) and tetrahydrohomofolate (homofolate-H_4).

The general approach of Boon and Leigh[1] for the synthesis of unambiguously 6-substituted 2-amino-4-hydroxypteridines was used to prepare XII. The N-phenyl-β-alanine derivative (I) was converted *via* II, III, IV, and V to the aminoketone (VI), the semicarbazone of which was condensed with 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine to give the intermediate (VII), after hydrolysis of the semicarbazone function. Hydrogenation of VII resulted in spontaneous cyclization of the ketone carbonyl with the formed 5-amino group to give the 7,8-dihydropteridine (VIII), $\lambda_{max}^{pH\ 13}$ 234 mμ (ε 21,200), 277 (7780), 330 (5180), that was oxidized to the pteridine (IX). Saponification gave chromatographically homogeneous "homopteroic acid" (X), $\lambda_{max}^{pH\ 13}$ 256 mμ (ε 26,900), 277 (21,900), 365 (7625). *Anal.*[2] Found: C, 54.7, H, 4.62; N, 25.6. The blocked derivative (XI), after reaction with diethyl L-glutamate by the mixed anhydride method followed by saponification, gave XII, $\lambda_{max}^{pH\ 13}$ 255 mμ (ε 24,600), 281 (19,500), 365 (7880). *Anal.* Found (for the hemihydrate): C, 51.2; H, 5.09; N, 21.2. On paper chromatography in 0.1 *M* $(NH_4)HCO_3$, 99% of the material was present in a single spot with R_f 0.89. The method of synthesis precludes a 7-substituted compound and, considering the ultraviolet spectra that support the pteridine structure, the analytical data, and the chromatographic behavior, there can be little doubt that homofolic acid has structure XII.



IX, R_1 = H; R_2 = Ac; R_3 = OEt
X, R_1 = R_2 = H; R_3 = OH
XI, R_1 = Ac; R_2 = CF_3CO; R_3 = OH
XII, R_1 = R_2 = H; R_3 = $NHCH(CO_2H)CH_2CH_2CO_2H$

(1) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).

Homofolate was converted to the dihydro derivative by dithionite reduction in 1 *M* 2-mercaptoethanol[3] and then was tested spectrophotometrically[4] as a substrate of dihydrofolate reductase from amethopterin-resistant mouse leukemia cells. Homofolate-H_2 was as effective a substrate as folate-H_2. The enzymatically formed homofolate-H_4 was not only completely inert as a participant in thymidylate synthesis but was a potent inhibitor of this enzyme. At 2.0×10^{-6} *M*, homofolate-H_4 caused a 50% inhibition of thymidylate synthetase from *E. coli*[5] in the presence of 80 times as much folate-H_4. Homofolate-H_4 analogs containing α-, β-, and γ-methyl glutamic acid moieties[6] were less inhibitory than homofolate-H_4.

Data obtained in a survey of the possible inhibitory action of homofolate-H_4 on a variety of tetrahydrofolate-requiring reactions indicate that thymidylate synthetase from *E. coli* appears to be the most sensitive of the enzymes tested (Table I).

In 0.006 *M* mercaptoethanol, homofolate-H_4 oxidized within 20 min., giving a compound with a spectrum closely resembling that of dihydrohomofolate. Addition of mercaptoethanol after oxidation did not reverse the reaction. Homofolate-H_4 bound formaldehyde mole for mole as does folate-H_4. In the presence of formaldehyde, homofolate-H_4 was stabilized over a period of 1 hr. at room temperature.

Homofolate is a poor growth inhibitor whereas homofolate-H_4 is a potent inhibitor of *Streptococcus faecalis* (ATCC 8043) and *Lactobacillus casei* (ATCC 7469, Table II). The inhibition is completely reversed for both organisms by thymidine (25 γ/ml.) even at levels of homofolate-H_4 140 times greater than that required for 50% inhibition. *Pediococcus cerevisiae* (ATCC 8081) is not affected by homofolate-H_4. This pattern of behavior is distinct from other antifolate agents.[7] Homofolate-H_2 surprisingly showed growth-factor activity for *S. faecalis*. It was about one-tenth as active as folate. This activity was not due to contamination with folate, as growth-promoting activ-

(2) Acceptable analytical data were obtained for all the solid substances encountered in the synthesis.

(3) M. Friedkin, E. J. Crawford, and D. Misra, *Federation Proc.*, **21**, 176 (1962).

(4) M. Friedkin, E. J. Crawford, S. R. Humphreys, and A. Goldin, *Cancer Res.*, **22**, 600 (1962).

(5) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, **237**, 3794 (1962).

(6) Substituted glutamates used in the synthesis of homofolate analogs were generously supplied by Dr. Alton Meister.

(7) R. L. Kisliuk, *Nature*, **188**, 584 (1960).