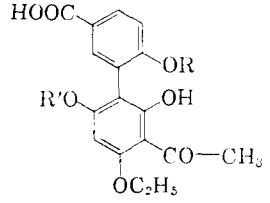
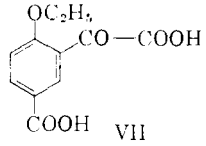
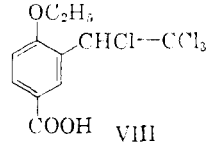
hinokiflavone,[4] sotetsuflavone pentaethyl ether produces 2,4-diethoxy-6-hydroxyacetophenone, *p*-ethoxybenzoic acid, and a phenolic acid (V), m.p. 257–258° (found: C, 63.89; H, 6.02. Calcd. for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92). V is not identical, by admixture, with a formerly reported acid (VI)[3], m.p. 258–259°, similarly obtained from sciadopitysin triethyl ether, and gives a monoketodicarboxylic acid (VII), m.p. 248–249° (dec.) (found: C, 55.79; H, 4.25. Calcd. for $C_{11}H_{10}O_6$: C, 55.46; H, 4.23) on oxidation with alkaline potassium permanganate solution. VII was identified with a synthetic sample, which was newly prepared by the mild oxidation of a condensation product (VIII),[5] through admixtures of itself and of its 2,4-dinitrophenylhydrazone, $C_{17}H_{14}O_9N_4$, m.p. 239–241°.



V. R = C_2H_5, R' = CH_3
VI. R = CH_3, R' = C_2H_5



VII



VIII

This degradative evidence leads to the assignment of structure IV for sotetsuflavone. Therefore, a ketoflavone obtained by the hydrolysis of sotetsuflavone must be represented by formula (IX) instead of II.

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RECEIVED DECEMBER 15, 1959

ASSOCIATION OF POLYMERS THAT REQUIRES ACTIVATION ENERGY[1]

Sir:

It is known that some groups, if present in polymeric molecules, may cause their reversible association in solution. Hydrogen bonding,[2] dipole interactions[3] or formation of clusters in polymeric salts[4] are responsible for these phenomena. For example, polystyrene endowed with the ——COO^-, Na^+ or ——CH_2O^-, Na^+ end groups associates in THF solution, while presence of the ——$CH(Ph)^- Na^+$ end-groups does not produce any detectable association.[5] These associations proceed very rapidly, in a second or two the system approaches closely the state of equilibrium if the viscosity of the solution is not too high.

A polystyrene endowed with the ——$CH(Ph)^-$, Li^+ end-groups has been prepared recently by polymerizing styrene in THF with lithium naphthalene. The initially fluid solution becomes gradually more and more viscous and a gel is formed after a day or two if the concentration is sufficiently high. The following experiment proved that this phenomenon is not due to a slow polymerization. A THF solution of polystyrene polymerized by lithium naphthalene was divided into three portions. By addition of water the organolithium compound was destroyed in the first portion shortly after its preparation, and at that time the solution was quite fluid. The second portion was kept at room temperature for 16 hours and then, when it was viscous, water was added. Finally, the last portion was kept for 40 hours and it was very viscous when the organolithium compound was destroyed by addition of water. After precipitation of the respective polymers it was shown that the polymerization was completed in each portion, and the intrinsic viscosities of the resulting polymers were identical, $[\eta] = 0.130$ in units g./100 cc.

The slowness of the association is astonishing and suggests that the process requires activation energy. This indeed seems to be the case. A solution of polystyrene polymerized by lithium naphthalene was divided into two portions. One was stored at −80°, while the second was introduced into a viscosimeter (a falling weight type). The time of falling slowly increased, being initially 9 sec., then 20 sec. after 12 hours, 40 sec. after 24 hours, and eventually 250 sec. after 48 hours. At that time the solution was very viscous, while the stored solution still remained fluid in spite of its low temperature. On the third day the stored solution was brought up to room temperature and then introduced into the same viscosimeter. Its initial viscosity corresponded again to 9 sec., and over a period of two days the viscosity increased to about 250 sec., the rate of increase being similar to that observed previously.

An explanation of this phenomenon is proposed. The polymeric ends might exist in 2 forms, *e.g.*, as covalent C–Li bonds and as ion pairs. Since each form represents a stable configuration, transformation of one form into the other must require activation energy. If the initially formed bond does not associate while the other does, then the observed association would show an apparent activation energy. This explanation is supported by another observation, namely, deepening of the color of the solution (from a bright red to a dark brown) with increase in the viscosity. Thus, the solution kept at −80° remained bright red and fluid; the color changed only after it was brought to room temperature and then left for two days. Alternatively it is possible that the activation energy is required for the reaction 2 —— $-C^-$, $Li^+ \rightarrow$ {——C, Li, C——}$^-$, Li^+, and it is hoped that future experiments will permit distinction between these alternatives.

(1) Research supported by Quartermaster Contract DA19-129-QM-1297, and by the National Science Foundation, G 5914.
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Whatever is the explanation, it seems that this is the first observed case of an association (other than coagulation of electrically charged colloidal particles) that requires an activation energy.

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GLUCOSE, A CONSTITUENT OF ALKALINE PHOSPHATASE[1]

Sir:

Alkaline phosphatase of swine kidney, found in the ribonucleoprotein particles of the microsomes[2] and released as an active fragment by proteolysis,[2,3] has been isolated in an apparently homogeneous condition by application of the procedures previously described[3] plus ion-exchange chromatography with Ecteola cellulose.[4] In a typical purification 2,000,000 units activity[2] with a specific activity of over 100,000 units per mg. total N (micro-Kjeldahl) was placed on a column of Ecteola 5 × 100 cm. and, after thorough washing with water, was eluted with a gradient of barium acetate at *p*H 9 varying from 0.01 to 0.05 *M* in 10 liters of solution. The active material was eluted symmetrically near 0.03 *M* and was concentrated by the barium procedure[3] to yield about 1,500,000 units material with a specific activity of 295,000 to 310,000 on the basis of total N. Rechromatography on Ecteola-cellulose, on Deae-cellulose[4] or on Dowex-2[3], paper electrophoresis and paper chromatography (ethanol–1 *M* ammonium acetate, 70–30) revealed no dissociation of absorbancy at 280 mμ from activity. The material was free of peptidase and diesterase activity when tested undiluted, amino acids were without effect on the activity and at no time was it possible to demonstrate dialyzable cofactors other than magnesium ion. The absorbancy in the ultraviolet was characteristic of protein with a maximum at 278 and a minimum at 250 mμ. However, in the course of treatment with dilute acid (0.1 to 1.0 *M* at 100°) the absorbancy was found to increase remarkably and, at the end of 2 hr., the absorbancy at 278 was nearly tripled. There was a parallel release of reducing material,[5] of ninhydrin reactive material,[6] and of material reacting with phosphomolybdate.[7] Paper chromatography (propanol–water, 80–20) separated a phosphomolybdate and ninhydrin reactive material from a ninhydrin negative but aniline hydrogen phthalate positive[8] (brown color) material with the same R_f as glucose. The untreated material in the cysteine methods of Dische[9] gave in the general reaction a product identical with the aldohexoses and in the secondary reaction of hexoses gave a product identical with glucose. A solution of alkaline phosphatase containing 30 μg. N per ml. after 2 hr. at 100° with 1 *N* HCl was found to contain 38 μg. glucose as determined by the reducing sugar method as determined by the cysteine reaction on the untreated material. The hydrolysate was found to contain approximately 25 μg. glucose as determined with glucose oxidase.[10]

The ninhydrin–phosphomolybdate positive material was found to migrate as a cation at *p*H 8 and to be unstable in more alkaline solutions. Other reactions of the compound were an immediate reaction with K_3FeCN_6 and $FeCl_3$ (blue) and an immediate reaction with K_3FeCN_6 alone (blue). Both these reactions were destroyed by previous treatment with traces of cupric ion with exposure to air. In a study of model compounds, phenolic compounds appear to have been eliminated but similar reactions have been observed with tri- and tetra-substituted pyrimidines; from the absorbancy in the ultraviolet, from studies of the model pyrimidines and from analogy with the proposed structure for vicine,[11] it is suspected that the material may be a diamino-5-hydroxypyrimidine attached, in the active material, to the glucose by a glycosidic linkage at the 5-hydroxy position.

(1) These studies were supported by grants from the U. S. Public Health Service. Detailed studies of the purification, the effects of amino acids and of divalent metal ions will be described by C. Lea.

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THE PHOSPHINOUS ACID $(CF_3)_2POH$ AND THE DIPHOSPHOXANE $(CF_3)_2POP(CF_3)_2$[1]

Sir:

We have recently isolated the new-type compounds $(CF_3)_2POH$ and $(CF_3)_2POP(CF_3)_2$ as stable liquids contrasting with the apparently complete instability of the corresponding hydrocarbon derivatives.[2] Evidently the highly electronegative CF_3 groups lower the power of phosphorus lone-pair electrons to bond either H^+ or $(CF_3)_2P^+$ coming from O. Thus these new $(CF_3)_2P$ compounds do not undergo the rearrangements $R_2POH \rightarrow R_2POH$ and $R_2POPR_2 \rightarrow R_2P\text{-}PR_2$ which probably represent the first stages of decomposition when R is a hydrocarbon group.

Synthesis and Characterization of the Diphosphoxane.—The reaction $2(CF_3)_2PI + Ag_2CO_3 \rightarrow CO_2 + 2AgI + (CF_3)_2POP(CF_3)_2$ (room temperature, repeated shaking with fresh silver carbonate) gave yields above 79%. The unused $(CF_3)_2PI$ (1%) was converted by AgCl to the easily

(1) This research was supported by the United States Air Force under Contract AF 33(616)-5435 (Subcontract No. 1) monitored by the Materials Laboratory, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio.

(2) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 144. We also have found that reactions expected to form $(CH_3)_2POH$ give nearly quantitative yields of $(CH_3)_2PH$ and $(CH_3)_2POOH$; and attempts to make $(CH_3)_2POP(CH_3)_2$ also give products suggesting disproportionation.