TABLE I

DIFFUSION OF ALKALINE PHOSPHATASE AT 5°

Cell no.	pH, 25°	Solvent	Enzyme: Source	Enzyme: Concentration, P.U./ml.	Enzyme: Specific activity, P.U./mg./P.N.	Diffusion coefficient, cm.² per day: Protein	Diffusion coefficient, cm.² per day: Enzyme
3	7.2	0.1 ionic strength phosphate buffer	Albers' horse kidney preparation[a]	584	250 approx.	0.0348	0.0238
3	6.9	0.1 *M* phosphate buffer also 0.5 *M* NaCl	Albers' horse kidney preparation	602			.0218
3	7.26	0.1 ionic strength phosphate buffer	Swine kidney[b]	1540	600		.0221
3	9.02	0.2 *M* veronal also 0.01 *M* DL-alanine	Swine kidney	1600	600		.0200
4	9.04	0.2 *M* veronal also 0.01 *M* DL-alanine	Swine kidney	1180	600		.0190
3	7.2	0.1 ionic strength phosphate buffer	Calf intestinal mucosa (Armour's)	120	111		.0161
3	7.2	0.1 ionic strength phosphate buffer	Albers' sheep kidney preparation[a]	178	77		.0180
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney[b]	2800	2500	.0236	.0170
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney	3230	1050	.0257	.0178
3	7.2	0.1 ionic strength phosphate buffer	Swine kidney	756	1050		.0163

[a] Ref. 1. [b] Ref. 5, 7.

described.[5,6,7] Swine kidney alkaline phosphatase preparations employed in this investigation are among the purest that have been reported. Protein was determined colorimetrically by the procedure of Lowry, *et al.*,[8] or by Kjeldahl.[9] Improved accuracy of the colorimetric determination of protein appeared to be obtained by carrying out the entire procedure at 25°. Twice crystallized ovalbumin was used as a standard protein. The concentrated ovalbumin solution was stored at −18° and contained 50% glycerol. Under these conditions it was adequately stable.

Diffusion coefficients for crystalline ovalbumin and crystalline swine pepsin were determined, giving values in accord with the literature.

Results and Discussion

Values obtained at 5° are compiled in Table I. Five additional experiments were carried out at 25° giving an average value of 0.038 cm.² per day. Molecular weight calculations from this latter figure are in fair agreement with those obtained at 5°. It can be seen that the diffusion coefficient ranges between 0.016 to 0.024 cm.² per day, irrespective of the solvent, *p*H, enzyme source, purity or concentration. In the last two experiments, in which the diffusion of protein was measured, it is apparent that protein is diffusing at a faster rate than enzyme, indicating the presence of impurities, but even so the values are in the same order of magnitude.

Molecular weight calculations from these data give values of 500,000 and over. Admittedly determinations in this molecular size range must be considered to be only approximations. Yet there is complete disagreement between our findings and those of Albers.[1] With this in mind, several experiments with commercial, graded porosity ultra-filters were carried out, and these, too, were indicative of a large molecular weight.

A point of interest is the similarity in results irrespective of the enzyme source. At least in this respect, it has not been possible to demonstrate significant differences between alkaline phosphatases from various sources.

The differential diffusion coefficient for several types of alkaline phosphatase has been determined using the diaphragm cell method.' An average value of 0.0192 cm.² per day was obtained at 5°. Molecular size estimations from these data, making the usual assumptions as to shape and density, are from 500,000 to 1 million, with the best estimation being 800,000. No significant difference was detected with respect to this property, between intestinal and kidney alkaline phosphatase, the latter enzyme being obtained from three species.

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(5) J. C. Mathies, *Biochim. Biophys. Acta*, **7**, 387 (1951); *Science*, **115**, 144 (1952).

(6) J. C. Mathies, E. D. Goodman and L. Palm, *Am. J. Physiol.*, **168**, 352 (1952).

(7) J. C. Mathies and E. D. Goodman, *Federation Proc.*, **11**, 255 (1952); Abstracts 123rd Meeting Am. Chem. Soc., pp. 24C (1953).

(8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(9) A. Hiller, J. Plazin and D. D. Van Slyke, *J. Biol. Chem.*, **176**, 1401 (1948).

Restricted Coupling in a Substituted Phenol[1]

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As part of the preparation of 4-arsonophenylazo derivatives of various phenols as potential carcinolytic agents, the coupling of diazotized *p*-arsanilic acid with 4-(1,1,3,3-tetramethylbutyl)-phenol (also commonly called octylphenol[3] or diisobutylphenol) (I) was studied. It was found that even though both positions in the phenol ortho to the hydroxyl group were unsubstituted, only one 4-arsonophenylazo group could be introduced even when an excess of the diazonium salt was used. This was somewhat surprising since tyrosine[4] couples with two moles of diazonium salt quite readily. Other 4-substituted phenols[5] also have shown coupling in both ortho positions.

It was necessary to add ethyl alcohol to the medium to keep the octylphenol in solution in alkali for the coupling reaction and it was thought that possibly the alcohol might have caused decomposition of enough of the diazonium salt to prevent coupling in both ortho positions. However, no phenylarsonic acid, the expected product from such decomposition, could be isolated from the reaction mixture. Furthermore, when the mono-substi-

(1) This work was aided by a grant to the University of Louisville from the Kentucky State Medical Research Commission.

(2) Student Summer Research Scholar, 1952.

(3) Kindly furnished by Rohm and Haas Co.

(4) H. Pauly, *Z. physiol. Chem.*, **94**, 284 (1915).

(5) L. Pauling, *et al.*, THIS JOURNAL, **64**, 2994 (1942).

tuted product was isolated, dissolved in alkali in the absence of alcohol and subjected to further attempts at coupling, apparently little, if any, additional coupling occurred. The arsenic content of the product isolated was only a little greater than that calculated for the mono-substituted product and far below that calculated for the di-substituted product. Chromatograms of an aqueous solution of the sodium salt of the product on an activated alumina column showed only one major band in the column on development with water.

Although neither expected nor, from a study of the structure, explainable to the authors, apparently the configuration of the monosubstituted product, 2-(4-arsonophenylazo)-4-(1,1,3,3-tetramethylbutyl)-phenol, is such that there is steric hindrance to the introduction of a second 4-arsonophenyldiazo group.

Experimental

Coupling of Octylphenol with Diazotized *p*-Arsanilic Acid.—To a solution of 2.37 g. of I (0.0115 mole) in 80 ml. of 5% sodium hydroxide and 50 ml. of 95% ethanol at 5° was slowly (1 hr.) added, with stirring, the solution of diazonium salt prepared from 5 g. of *p*-arsanilic acid (0.023 mole) in the usual manner. The resultant blood-red solution was placed in the refrigerator overnight. The product, a red-orange solid, was precipitated by addition of concd. hydrochloric acid, filtered, redissolved in 5% sodium hydroxide, extracted three times with ether, reprecipitated with concd. hydrochloric acid, washed with water and dried at 110° overnight; yield 2.2 g. (43%).

Anal. Calcd. for $C_{20}H_{27}AsN_2O_4$: As, 17.3. Calcd. for $C_{26}H_{32}As_2N_4O_7$: As, 22.7. Found: As, 17.8, 17.5, 17.0, 17.4.

A portion of the product was dissolved in 5% sodium hydroxide, treated with excess diazotized *p*-arsanilic acid and worked up in the manner described above. The product isolated was a red-orange solid.

Anal. Found: As, 18.0.

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The Actinide–Lanthanide Analogy as Exemplified by Solvent Extraction Behavior

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Interest in the actinide hypothesis has been revived in recent years, and due in large part to the studies of Seaborg and co-workers,[1] who have correlated a large mass of data pertaining to the lanthanides(III) and to the elements(III) of atomic numbers 89–98 and successfully applied the hypothesis in devising separations procedures for the trivalent transuranic elements, the term actinide has had wide acceptance as a generic term for those elements of atomic number ranging from 89 (actinium) to 98 (californium, the element of highest atomic number known). However, the wide variation, even though it is a regular one, within this grouping of elements with respect to the most stable valence state in aqueous solution has led to considerable difference of opinion, and Bouissieres and Haissinsky[2] consider the actinide hypothesis untenable. For the purpose of pointing out the similarities between representatives of this grouping of elements, in the trivalent state, and trivalent lanthanides the following comparison of their solvent extraction behavior is presented. This analogy may be compared with the well-documented analogy with respect to ion-exchange behavior.[3]

Distribution ratio studies were performed in a manner described previously,[4] using β-active 40-h La^{140}, 275-d Ce^{144}(III), 2.6-y Pm^{147}, *ca.* 5.4-y $Eu^{152,154}$(III) and 6.1-h Ac^{228} and α-active 24,100-y Pu^{239}(III), *ca.* 500-y Am^{241}(III), and 150-d Cm^{242}.[5] So that a comparison of relative behavior should be unambiguous, Pm was present in all solutions involved in obtaining Pu, Am and Cm data, and Am was present in all solutions involved in obtaining lanthanide and Ac data. Consequently, each set of measurements had an internal standard. For example, a set of Pu–Am–Cm data was discarded as internally inconsistent unless the K of Pm throughout the set had a constant value within 2%.

A set of self-consistent Pu–Am–Cm data having been established, data for Ac were obtained depending upon Am as the internal standard, etc. Radiometric assays were performed as described previously,[4] aluminum absorbers being used to eliminate α-interference in β-counting. It should be noted that for purposes of this discussion the distribution ratio, K, is considered to be equal to the ratio of measured radioactivities associated with equal aliquots of the equilibrated organic and aqueous phases, respectively.

The results of these experiments show that log K is a linear function of atomic number (at least in the region studied)[5] for both actinides(III) and lanthanides(III) using tri-*n*-butyl orthophosphate,[6] $(n\text{-}C_4H_9O)_3PO$, as the organic phase and 15.6 M HNO_3, 12.0 M HNO_3 or 12.0 M HCl as the aqueous phase. This relationship has been reported previously[4] for the lanthanides in a tributyl phosphate–nitric acid system.

Likewise, the nitric acid dependency of K for Am is found to parallel closely that of K for Pm. The crossing of the acid-dependency curves for Am and Cm has been demonstrated, the crossing of these curves being reminiscent of the crossing of the corresponding curves for two lanthanides or for Pm and Y.[4]

(1) Among several pertinent publications may be mentioned: G. T. Seaborg, *Chem. Eng. News*, **23**, 2192 (1945); K. Street, Jr., and G. T. Seaborg, THIS JOURNAL, **72**, 2187 (1949); G. T. Seaborg, R. A. James and L. O. Morgan, Natl. Nuclear Energy Ser., Div. IV, **14B**, Transuranium Elements, Pt. II, 1525 (1949); G. T. Seaborg, R. A. James and A. Ghiorso, *ibid.*, p. 1554; and L. B. Werner and I. Perlman, *ibid.*, p. 1586.

(2) G. Bouissieres and M. Haissinsky, *Bull. soc. chim. France*, [5] **18**, 557 (1951).

(3) K. Street, Jr., and G. T. Seaborg, ref. 1.

(4) D. F. Peppard, J. P. Faris, P. R. Gray and G. W. Mason, *J. Phys. Chem.*, **57**, 294 (1953).

(5) The authors were unable to stabilize the trivalent state of Pu in the nitric acid systems studied, and Np(III) could not be studied in either the nitric acid or hydrochloric acid systems. In other studies, the absence of Ce(IV) was assured by the addition of a small quantity of hydrogen peroxide to the feed make-up at 60°; and Pu(III) was obtained by reduction of Pu in any combination of valence states with ferrous ion. All of the other tracers used were automatically obtained in the trivalent state by evaporation with nitric acid or hydrochloric acid.

(6) Tri-*n*-butyl orthophosphate, obtained from Commercial Solvents Corporation, was washed with several portions of 10% aqueous sodium carbonate prior to acid pre-equilibration to assure the absence of phosphoric acid, $(C_4H_9O)(HO)_2PO$ and $(C_4H_9O)_2(HO)PO$.