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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RECEIVED JULY 30, 1953

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 $(C_4H_9O)_2(HO)PO$.

The distribution ratio for Am has been shown to have an approximate third-power dependence on the tributyl phosphate concentration, expressed as volume per cent. In this, also, Am behavior parallels that of Pm.

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Coprogen, the Isolation of a New Growth Factor Required by *Pilobolus* Species

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RECEIVED AUGUST 10, 1953

The complex nutrition of the genus *Pilobolus* has been described by several investigators.[1,2] These workers found it necessary to add dung or dung extracts to their culture medium. In a general survey of the distribution of the factor(s) that was essential for the growth of *Pilobolus kleinii*, it was found that the dung extracts could be replaced by the fermentation liquors of a number of species of bacteria and fungi. The isolation of a crystalline, biologically-active compound from such sources has been announced.[3] This substance has been designated "Coprogen" because of its ability to stimulate the growth of the *Coprophyllic* fungi. The method of assay and the general cultural and nutritional characteristics of the genus *Pilobolus* has also been described.[3,4]

Hesseltine and co-workers[4] listed a number of microörganisms that had been tested for their ability to produce the factor essential for *Pilobolus kleinii*. Culture *Penicillium* sp. appeared to be the most feasible organism for large scale production of the growth substance and was, therefore, used to produce adequate amounts of the compound for isolation.

The purification of the compound was initially done by solvent extraction, adsorption and elution from florisil and partition chromatography. Later procedures eliminated the use of the florisil adsorption step.

Crystallization of the active compound was effected by dissolving the active lyophilized fraction from the partition chromatogram in absolute ethanol. On standing a brick-red, crystalline compound separated. If only a slight trace of moisture was present, the compound tended to hydrate and precipitated in an amorphous form. The crystalline material is practically insoluble in ethanol and, for recrystallization, the compound must be dissolved in water, lyophilized and then again crystallized from ethanol.

Elemental analysis of the compound indicated the presence of carbon, hydrogen, nitrogen, oxygen and iron. The organo-iron nature of the compound and the fact that certain metallo porphyrins are growth factors for microörganisms[5-8] suggested that perhaps Coprogen was related to the porphyrin compounds. The absorption spectra and chemical properties of the isolated compound ruled against this possibility.

Coprogen exhibited a broad absorption maximum at 440 mμ with an $E^{1\%}_{1\ cm.}$ of 36.6 in 50% ethanol. The Soret band which is characteristic of heme compounds was clearly missing.

When Coprogen was dissolved in dilute alkali, ferric hydroxide precipitated and the ultraviolet absorption spectrum of the compound was destroyed. Concomitantly, the biological activity of the compound also was destroyed.

Experimental

Assay Methods.—Initially, the activity of the various fractions was determined by the plate assay method. In subsequent work, *Pilobolus kleinii* was cultured in liquid media and the weight of the mycelia was used as a measure of growth.[4]

Fermentation.—Culture *Penicillium* sp. was cultured in large scale fermentation apparatus equipped for agitation, aeration and temperature control. The medium contained 1% Bacto peptone, 0.1% ammonium sulfate, 0.1% sodium acetate and 0.1% potassium dihydrogen phosphate. Tap water was used throughout. After inoculation the fermentation was continued for three days at a temperature of 26–28° and with an aeration rate of 1 volume of air per volume of medium per minute. Filter-cel (0.1%) was added and the mycelium and Filter-cel was removed in a filter press.

Solvent Extraction.—To the clear filtrate was added 500 g. of ammonium sulfate per liter. The solution was then extracted twice with one-quarter volumes of butanol. The butanol extracts were combined and then concentrated under reduced pressure with the slow, constant addition of water. The distillation was continued until the butanol was completely removed and only a small volume of water remained.

The solution was filtered to remove any suspended matter and then extracted with approximately four one-quarter volumes of benzyl alcohol. The benzyl alcohol extracts were combined and then extracted with a one-tenth volume of water which removed inorganic salts and other impurities. Two volumes of ether or ethyl acetate were added to the combined benzyl alcohol extracts and the solution was then extracted with several one-quarter volumes of water. The activity and color were almost quantitatively extracted into the aqueous phase. The aqueous extracts were combined, concentrated to remove solvents and then lyophilized. The dried product was orange-brown in color, stable for storage and very convenient to handle.

Partition Chromatography.—"Celite 545"[9] (50 g.) was mixed with 25 ml. of the aqueous phase of a mixture of butanol:ethyl acetate:0.01 *N* hydrochloric acid (2:1:1). The moist "Celite" was packed into a column 2 cm. i.d. × 60 cm. Five hundred mg. of the crude lyophilized material was dissolved in 25 ml. of the solvent phase. This solution was placed in the column and immediately followed by fresh solvent.

If the column was extruded after the solvent front had reached the end of the column, several well-defined zones were evident. The first zone was purple and gave a strong $FeCl_3$ test. The second zone was orange, reacted strongly positive with $FeCl_3$ and contained Coprogen. The third and fourth zones were white and pale purple, respectively, and both zones gave a strong $FeCl_3$ test.

A flowing chromatogram was used and the fraction containing the orange band was collected, neutralized with sodium hydroxide and then concentrated under vacuum.

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