repulsion between the entering negatively-charged thiolate ion and the like-charged carboxylate group overshadowing the similar repulsion between the thiolate ion and the pair of electrons it is displacing from the acetylenic group. This latter repulsion seems to be a satisfactory explanation for the usual *trans* nucleophilic additions.

This argument leads to the prediction that similar nucleophilic additions to acetylenes bearing negatively charged substituents should also proceed *cis* rather than *trans*. It is gratifying to note that there is reasonable evidence for a *cis* addition of ammonium sulfite to salts of propiolic acid to form *trans*-2-sulfoacrylic acid.[4]

The above explanation for *cis*-nucleophilic addition of *p*-toluenethiol to sodium propiolate is supported by the observation that such additions to the two acetylenes, ethyl propiolate and benzylacetylene (bearing similarly electronegative but *uncharged* substituents), proceed in the normal *trans* fashion, the products being ethyl *cis*-2-*p*-tolyl-mercaptoacrylate and *cis*-1-benzoyl-2-*p*-tolylmercaptoethene, respectively. Saponification of the former product gave a compound identical with the minor product obtained by like treatment of sodium propiolate.

We hope soon to develop information regarding whether or not nucleophilic addition can be forced to proceed *cis* as a result of *steric* factors as well as electronic factors. Also, data relating to the detailed mechanism of a *trans* nucleophilic addition will be forthcoming.

(4) H. J. Backer and A. E. Beute, *Rec. trav. chim.*, **54**, 523 (1935).

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A NEW ADENYL-SUCCINIC ACID DERIVATIVE CONTAINING SULFATE AND A PEPTIDE[1]

Sir:

Recently, the occurrence of adenine-succinic acid and adenyl-succinic acid has been reported from several laboratories. Yeast,[2] *Escherichia coli*,[3] mammalian liver[4] and cod liver[5] have been demonstrated to contain one or both of these compounds. In the present communication the authors wish to report the isolation and identification of a derivative of adenylsuccinic acid (I) from salmon liver.

The livers were excised from live spring (king) salmon at sea and immediately frozen in dry ice. The acid soluble phosphorus compounds were extracted into cold perchloric acid and chromatographed on Dowex-1 anion exchange resin exactly as previously described except that a refrigerated column and fraction collector were used.[6] The fraction under consideration (E) appeared immediately after adenosine diphosphate from the ion exchange column in a formic acid system (Fig. 1). Fraction E was separable into three components

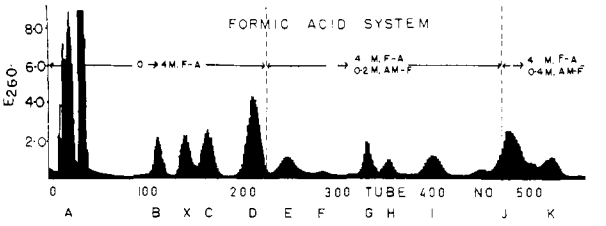
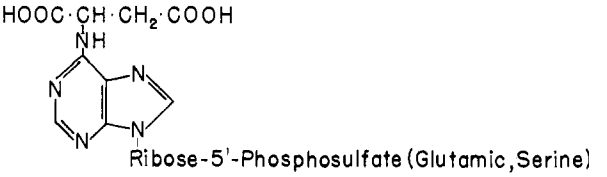


Fig. 1.—Gradient elution of nucleotides on a 1.0 × 20 cm. bed of Dowex-1 formate resin at 0°. The mixing volume was 500 ml. and 5-ml. fractions were collected.[6]

(E_1, E_2 and E_3) by paper chromatography (Table I). Compound I (E_2) moved centrally in relation to the other two and generally comprises approximately 80% of the fraction. It behaved as a single entity on several paper chromatograms (Pabst solvents 1, 2 and 3) and was electrophoretically homogeneous at several *p*H's. Compound I has an absorption maximum of 266 mμ in acid and gives positive tests for phosphate, sulfate and ribose, and a positive ninhydrin reaction. Analytical data on I shows an approximately equimolar ratio of adenine succinate, total P, ribose, sulfate, and *cis*



glycol (Table II). Acid hydrolysis (1.0 *N* HCl) of I for 10 minutes at 100° released a compound with an R_f identical to ribose-5′-phosphate (R-5′-P) and an ultraviolet absorbing compound (E_4). Substance E_4 showed no diazotizable amine,[7] was free from ribose phosphorus and sulfur and behaved similarly to adenine-succinic acid upon paper electrophoresis at *p*H 3.3 and 6.0.[4] Formic acid hydrolysis of E_4 followed by paper chromatography in several solvent systems yielded adenine, hypoxanthine (minor component), aspartic and fumaric acids, thus completing the identification of E_4 as adeninesuccinic acid.

Since periodate oxidation[8] of I showed a free *cis* glycol, and since R-5′-P was obtained by acid hydrolysis, the phosphate must be attached to the 5 position of ribose. Very mild acid hydrolysis (0.01 *N* HCl for 1 hr. at room temperature) of I produced a peptide and sulfate containing nucleotide which were separable chromatographically. Further treatment of the nucleotide in 0.01 *N* HCl for 10 minutes at 100° liberated inorganic sulfate and adenyl-succinic acid. The sulfate therefore must be attached to the phosphate. Compound I decomposes unless a refrigerated column is used. This suggests the possibility that adenyl succinate isolated from

(1) Presented in part at the 130th Meeting of the American Chemical Society, Atlantic City, September, 1956, but not abstracted.
(2) C. E. Carter and L. H. Cohen, THIS JOURNAL, **77**, 499 (1955).
(3) I. Lieberman, *ibid.*, **78**, 251 (1956).
(4) W. K. Joklik, *Biochem. Biophys. Acta*, **22**, 211 (1956).
(5) I. D. E. Storey and D. N. Love, *Biochem. J.*, **64**, 53P (1956).
(6) R. B. Hurlbert, H. Schmitz, A. F. Brumm and V. R. Potter, *J. Biol. Chem.*, **209**, 23 (1954).
(7) J. M. Ravel, R. E. Eakin and W. Shive, *J. Biol. Chem.*, **172**, 67 (1948).
(8) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).

other natural products by ion exchange chromatography is a decomposition product of a larger molecule.

TABLE I

PAPER CHROMATOGRAPHY OF FRACTION E

Solvent systems: 1–8, 11 isobutyric/NH_4OH/H_2O 66/1/33; 9, 10 ethanol/NH_4OH/H_2O (8:1:1); 12, 13 ethanol/NH_4OH (95:5); AMP = adenylic acid, ADP = adenosine diphosphate, ATP = adenosine triphosphate, R-P = ribose-phosphate.

		R_f		
		Ultra-violet	Ninhydrin	Phos-phate
1	5′-AMP	0.47		
2	E_1	.05		
	E_2	.15	0.15	
	E_3	.40		
3	5′-ATP	.18		
4	5′-ADP	.23		
5	E_4	.44		0.17
6	5′-RP			.17
7	E_4 (formic hydrolysis)	.84	.23	
		.52		
8	Adenine	.83		
	Hypoxanthine	.51		
	Aspartic		.24	
9	Fumaric	.21	.21	
10	E_4 (formic hydrolysis)	.21	.21	
11	E_2 (10 min., .001 *N* HCl, 100°)	.17	.13	
12	Glutamic		.02	
	Serine		.14	
13	Peptide (hydrolysed)		.02	.14

TABLE II

ANALYSIS OF COMPOUND I

Adenine succinic acid was determined by absorption at 266 mμ using E 16.9 × 10^3 at *p*H 1.0, ribose by the orcinol method, labile phosphorus by 7 min. hydrolysis in 1 *N* acid, total phosphorus by $HClO_4$ digestion, the color in both cases was developed by the Gomori method. Vicinal glycol was estimated spectrophotometrically.[8] Sulfate was determined colorimetrically after conversion to H_2S,[9] which then reacted with *p*-phenylenediamine to form Lauth's violet.[10]

	Moles
Adeninesuccinic acid	1.00
Ribose	1.12
Labile-P	0.00
Total P	1.05
Sulfate	1.37
cis Glycol	1.27

When I was subjected to mild acid hydrolysis (0.01 *N* HCl, 100°, 10 min.) and chromatographed in several systems, a single ninhydrin position spot was obtained which gave glutamic acid and serine in equal concentrations following hydrolysis in 6*N* HCl for 12 hours at 120°. It has not yet been established whether I contains a dipeptide or polypeptide of glutamic acid and serine. Fraction E from another liver preparation contained glycine so this portion of the molecule is probably a variable. The manner of linkage of the peptide to the nucleotide and the sequence of the amino acid residues in the peptide chain are under further investigation.

(9) C. L. Luke, *Anal. Chem.*, **21**, 1369 (1949).
(10) D. S. C. Polson and J. D. H. Strickland, *Anal. Chim. Acta*, **6**, 452 (1952).

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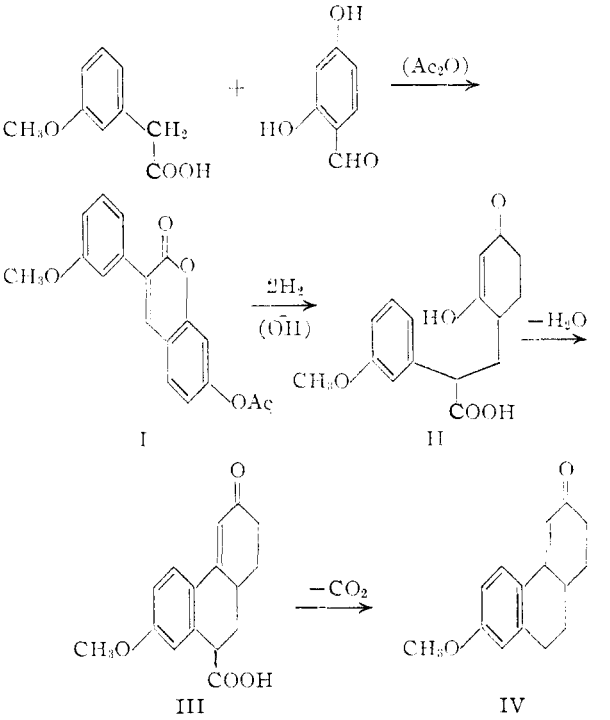
SYNTHESIS OF 1,2,3,9,10,10a-HEXAHYDRO-3-keto-PHENANTHRENES FROM 3-ARYL-7-ACETOXYCOUMARINS

Sir:

To the growing collection of methods for elaborating polycyclic compounds we now add a new sequence of reactions leading to some important hydrophenanthrene ketones.



Features contributing to the success of this method are (1) improved Perkin condensation of methoxyphenylacetic acids with β-resorcylaldehyde and other phenolic carbonyl compounds, (2) novel and very efficient hydrogenation and *in situ* hydrolysis of resulting coumarins, such as I, incorporating a resorcinol unit, in the presence of *palladium* and dilute alkali, to dihydroresorcinols such as II, and (3) cyclization of II with polyphosphoric acid. Dehydration of II to III evidently depends for its success upon factors similar to those at work in related reactions[1,2] involving attack of cyclic 1,3-keto-enols upon the aromatic ring, and in this case may involve intermediate enol lactone formation.

Compound I, m.p. 145–146° (Found: C, 69.5; H, 4.54; $\lambda_{max}^{chf.}$ 5.67 and 5.80 μ), was prepared in 66% yield by condensation of 2,4-dihydroxybenzaldehyde and 3-methoxyphenylacetic acid, using

(1) A. J. Birch and H. Smith, *J. Chem. Soc.*, 1882 (1951).
(2) G. N. Walker, THIS JOURNAL, **78**, 2340, 3201 (1956).