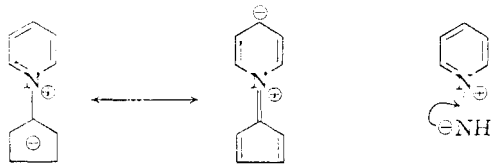
The proposal of back coördination into a pyridine ring is new in the area of boron hydride chemistry. However, several systems common to carbon chemistry apparently exhibit this phenomenon. One excellent example of such an interaction is presented by the red-gold compound I, recently described by Kosower and Ramsay.[24]



I II

The conjugate base of the N-aminopyridinium ion II is blue-violet[25] and constitutes another example of such back coördination.

Experimental

Materials.—Decarborane was sublimed before use. All of the substituted pyridines except the *p*-methoxy derivative were obtained from either Columbia Organic Chemical Co. or Aldrich Chemical Co. and were used without further purification. Both pyridine and quinoline (Eastman Chemical Co.) were distilled before use. Acetonitrile was Eastman Spectrograde.

Preparation of *p*-Methoxypyridine.—The *p*-methoxypyridine was prepared from sodium methoxide and *p*-chloropyridine in essentially the same way as reported for the synthesis of the *o*-isomer.[26] The product had a boiling point of 98–100° at 38 mm. pressure and a refractive index of $n^{25}D$ 1.5184. These compare favorably with the reported constants[27] b.p. 102° (40 mm.) and $n^{25}D$ 1.5176. The infrared spectrum was also consistent for that expected for *p*-methoxypyridine.

Preparation of 6,9-Bis-diethyl Sulfide Decarborane.—In a 500-cc. round-bottom flask, equipped with condenser, were placed 45 g. of sublimed decaborane (0.34 mole), 100 ml. of dry benzene and 120 ml. of ethyl sulfide. The mixture was refluxed under dry nitrogen for about 3 hours or until no more gas was evolved. The yellow solution was cooled to room temperature and the product crystallized out by adding ether and pentane. After cooling, the fluffy crystals were filtered and washed with ether; m.p. 90–91°. The total yield was 85 g. (83%).

Anal. Calcd. for $B_{10}C_8H_{32}S_2$: B, 36.07; C, 31.98; H, 10.66. Found: B, 34.97; C, 32.43; H, 11.02.

Preparation of 6,9-Bis-pyridine Decaborane Derivatives.—All of the pyridine decaborane derivatives were prepared in the same manner with the various yields and results listed in Table I. The compounds were recrystallized from large volumes of hot acetonitrile. A typical example of the general procedure in these preparations is listed below for the parent compound.

6,9-Bis-pyridine Decaborane.—In 40 ml. of benzene were dissolved 3 g. (0.01 mole) of bis-diethyl sulfide decaborane and 2 g. (0.025 mole) of pyridine. The solution was stirred under an atmosphere of nitrogen at room temperature for about 4 hours. The yellow solid which separated from solution was filtered and dried; yield 2.3 g. (82%). Recrystallization from much acetonitrile afforded small yellow crystals.

Ultraviolet Spectra.—The spectra of the 6,9-bis-pyridine decaborane derivatives were measured with a model DK-1 Beckman spectrophotometer. Samples were weighed out accurately on a micro-balance and diluted with acetonitrile to a 10-ml. volume to give approximately a 10^{-4} *M* solution.

Infrared Spectra.—A model 137 Perkin–Elmer Infracord was used to obtain Nujol mull infrared spectra of the pyridine derivatives.

Acknowledgments.—The authors wish to thank Mr. R. D. Strahm for the ultraviolet spectra and Mr. J. Sanford for the elemental analyses. We are also indebted to Dr. Hans Jonassen for the magnetic susceptibility measurements which showed these compounds to be diamagnetic. This work was performed under the sponsorship of the U. S. Army Ordnance Corps, Contract No. DA-01-021-ORD-11878.

(24) E. M. Kosower and B. G. Ramsey, *J. Am. Chem. Soc.*, **81**, 856 (1959).

(25) Reported by R. Huisgen at the Robert A. Welch Foundation Conference on Molecular Structure and Organic Reactions, Houston, Tex., April, 1960, and suggested to the authors by Prof. R. B. Woodward.

(26) T. B. Grave, *J. Am. Chem. Soc.*, **46**, 1466 (1924).

(27) D. G. Leis and B. C. Curran, *ibid.*, **67**, 79 (1945).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON 6, WISC.]

Studies on the 4-Hydroxycoumarins. XVII.[1a] The Resolution and Absolute Configuration of Warfarin[1b]

BY BRUCE D. WEST, SEYMOUR PREIS, COLLIN H. SCHROEDER AND KARL PAUL LINK

RECEIVED FEBRUARY 9, 1961

The anticoagulant *rac*-warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] has been completely resolved through its quinidine and quinine enolates. The levorotatory (in aqueous alkali) form is about seven times more active in the rat than its enantiomer. (−)-Warfarin and (+)(S)-β-phenylcaproic acid have been converted by reactions not involving the asymmetric centers to the two enantiomers of 1,1-di-(*o*-anisyl)-3-phenylhexan-1-ol. The (S) configuration has been assigned to (−)-warfarin.

The anticoagulant *rac*-warfarin [3-α-(acetonylbenzyl)-4-hydroxycoumarin] was synthesized here by the Michael condensation of 4-hydroxycoumarin with benzalacetone.[2] In 1948, it was proposed for rodent control and rapidly gained world-wide use. In 1952, its water-soluble sodium derivative was introduced for clinical use. It now rivals Dicumarol® [3,3′-methylenebis-(4-hydroxycoumarin)], through which oral anticoagulation was made possible.[3]

Resolution of *rac*-Warfarin.—Attempts to form covalent diastereoisomers with the resolving agents *l*-menthoxyacetyl chloride,[4] *l*-menthydrazide[5] and

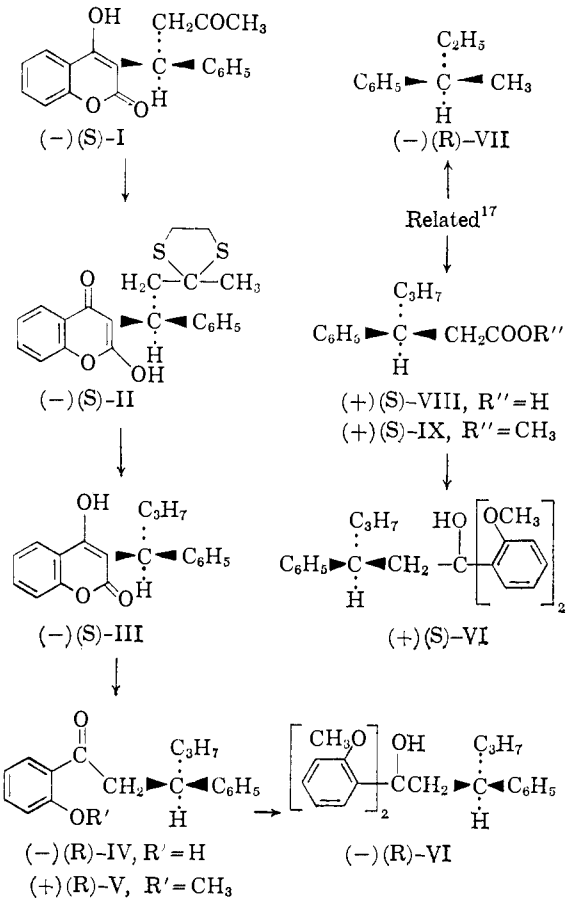
(1) (a) Previous paper in this series: S. Roseman, C. F. Huebner, R. Pankratz and K. P. Link, *J. Am. Chem. Soc.*, **76**, 1650 (1954). (b) Published with the approval of the Director of the Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) M. Ikawa, M. A. Stahmann and K. P. Link, *J. Am. Chem. Soc.*, **66**, 902 (1944).

(3) K. P. Link, *Circulation*, **19**, 97 (1959).

l-menthyl bromoacetate[6] yielded unsatisfactory products. *d*-Camphor-10-sulfonyl chloride[7] yielded a crystalline ester which apparently existed as a "diastereo compound."[8]

The acid strength of warfarin (pK_a 4.8)[9] permits formation of stable alkaloid salts. However, attempts to form crystalline salts with quinine, strychnine, cinchonine, cinchonidine, brucine and nicotine were unsuccessful. The quinine methohydroxide-warfarin salt was crystalline, but after 17 recrystallizations it was not optically pure.[10] Quinidine, however, formed a readily purified salt with *rac*-warfarin and (S)-warfarin[11] was obtained in good yield. The warfarin-quinidine salts rich in (R)-warfarin could be obtained only as glasses. Decomposition of these glasses yielded (R)-warfarin of 60–90% optical purity. Because (R)-warfarin has a crystal habit different from that of *rac*-warfarin, it could be obtained by fractional crystallization. The more soluble (R)-warfarin, which deposited as large crystals, was separated manually from the fine needles of optically impure warfarin. Reconstitution of pure (R)-warfarin-quinidine salt also yielded a glass. Subsequently it was found that the optically impure (R)-warfarin could be purified easily through a crystalline quinine salt.



Studies made here by Dr. T. H. Lin and Dr. W. F. Blatt have shown that the two enantiomers of warfarin have different anticoagulant potency. (S)-Warfarin is about 7 times more active in the rat than (R)-warfarin, as indicated by the prolongation of the prothrombin time caused by single doses[12] or by the multiple dose procedure to induce fatal hemorrhage.[13]

Relation of (−)(S)-β-Phenylcaproic Acid to (−)(S)-Warfarin.—Kenyon, *et al.*,[14] showed that the product from the action of phenylmagnesium bromide on the *p*-toluenesulfonate of (+)(S)-2-butanol[15] was (−)-2-phenylbutane [(−)-VII]. Since this reaction (SN2) proceeds with inversion,[14,16] the configuration of (−)-2-phenyl-butane can be designated (R). Levene and Marker[17] related (+)-β-phenylcaproic acid [(+)(S)-VIII] to (−)-2-phenylbutane [(−)(R)-VII].

(−)(S)-Warfarin [(−)(S)-I] was reduced by Raney nickel desulfurization of its ethylene thioketal [(−)(S)-II] to (−)(S)-3-(α-phenylbutyl)-4-hydroxycoumarin [(−)(S)-III]. Experience in this Laboratory has shown that decarboxylation of 4-hydroxycoumarins is best effected by weak alkali. In this case, decarboxylation of (−)(S)-III was accomplished by simply refluxing an aqueous solution (*ca.* *p*H 9) of its sodium enolate. The (−)(R)-*o*-hydroxy-β-phenylcaprophenone [(−)(R)-IV] obtained was an optically active liquid. It was O-methylated and the product, (+)(R)-*o*-methoxy-β-phenylcaprophenone [(+)(R)-V], was treated with *o*-anisylmagnesium bromide to yield the tertiary alcohol (−)(R)-1,1-di-(*o*-anisyl)-3-phenylhexan-1-ol [(−)(R)-VI].

Treatment of the methyl ester derived from partially resolved (+)(R)-β-phenylcaproic acid [(+)(R)-VIII] with excess of *o*-anisylmagnesium bromide introduced two anisyl groups to yield (+)(S)-VI, the enantiomer of the alcohol obtained from (−)-warfarin. This shows that (−)-warfarin is related to (−)-β-phenylcaproic acid and has the (S) configuration. The stereochemical relationships leading to this assignment are shown.

Experimental

Quinidine-(S)-warfarin Salt and (S)-Warfarin [(−)(S)-I].—A mixture of 2 l. of chloroform and 3 l. of acetone containing 324 g. (1.0 mole) of quinidine and 308 g. (1.0 mole) of *rac*-warfarin was warmed to effect solution. A crystalline product separated and the solution was held at −10° overnight. The quinidine-warfarin salt, filtered from the solution (filtrate A), weighed 241 g., $[\alpha]^{24}D$ + 92° (*c* 1.8, 95% ethanol). A second crystallization from 3.5 l. of acetone yielded 153 g. of pure quinidine-(S)-warfarin salt, $[\alpha]^{25}D$ + 87° (*c* 1.7, 95% ethanol). The quinidine-(S)-

(4) A. W. Ingersoll in R. Adams, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 381.

(5) Woodward reagent [R. B. Woodward, T. P. Kohman and G. C. Harris, *J. Am. Chem. Soc.*, **63**, 120 (1941)] was tried by E. A. Popenoe, Jr. (Ph.D. Thesis, University of Wisconsin, 1950).

(6) S. Preis, Ph.D. Thesis, University of Wisconsin, 1957.

(7) J. Read and W. J. Grubb, *J. Chem. Soc.*, 188 (1930).

(8) R. A. Kloss, Ph.D. Thesis, University of Wisconsin, 1956.

(9) J. A. Snyder, Ph.D. Thesis, University of Wisconsin, 1953.

(10) J. A. Snyder, unpublished observations.

(11) We are using the absolute configuration nomenclature proposed by R. S. Cahn, C. K. Ingold and V. Prelog [*Experientia*, **12**, 84 (1956)].

(12) H. A. Campbell, W. K. Smith, W. L. Roberts and K. P. Link, *J. Biol. Chem.*, **138**, 1 (1941).

(13) R. S. Overman, J. B. Field, C. A. Baumann and K. P. Link, *J. Nutrition*, **23**, 589 (1942).

(14) J. Kenyon, H. Phillips and V. Pittman, *J. Chem. Soc.*, 1080 (1935).

(15) J. A. Mills and W. Klyne in W. Klyne, "Progress in Stereochemistry," Vol. I, Butterworth, London, 1954, p. 183.

(16) D. J. Cram, *J. Am. Chem. Soc.*, **74**, 2150 (1952).

(17) P. A. Levene and R. E. Marker, *J. Biol. Chem.*, **97**, 565 (1932).

warfarin salt was soluble in acetone to the extent of 5 mg./ml. at −10° and 16 mg./ml. at 25°. There was no change in rotation upon recrystallization from acetone or tetrahydrofuran.

Anal. Calcd. for $C_{39}H_{40}N_2O_8 \cdot H_2O$: C, 71.98; H, 6.55; N, 4.36. Found: C, 72.4; H, 6.6; N, 4.1.

The salt was decomposed by partition between 1 l. of 0.5*N* sodium hydroxide and 0.5 l. of chloroform. The aqueous layer was added to excess hydrochloric acid and 70 g. of (S)-warfarin was collected.

Crystallization at room temperature from 600 ml. of warmed 80% aqueous acetone yielded 25 g. of (S)-warfarin, m.p. 172–173°, $[\alpha]^{25}D$ −148.0 ± 0.5° (*c* 1.2, 0.5 *N* sodium hydroxide), −25.5 ± 1° (*c* 2, acetic acid), −15.5 ± 1° (*c* 3, acetonitrile), + 15.7 ± 1° (*c* 3.7, butanone-2) and +19.1 ± 1° (*c* 1.1, propanol-2). Addition of 100 ml. of water to the filtrate and cooling resulted in a second crop of 32 g. of (S)-warfarin, m.p. 171–173°, $[\alpha]^{25}D$ −148.0 ± 0.5° (*c* 1.2, 0.5 *N* sodium hydroxide).

(S)-Warfarin Sodium.—An excess of (S)-warfarin was stirred with 7 ml. of 0.5 *N* sodium hydroxide solution. Lyophilization of the filtrate yielded 1.1 g. (S)-warfarin sodium, $[\alpha]^{24}D$ −95.8 ± 0.5° (*c* 3.2, 95% ethanol).

(R)-Warfarin [(+)(R)-I].—The filtrate A from above was concentrated to 1 l. and diluted with 1 l. of acetone. Upon cooling, 96 g. of quinidine-warfarin salt, $[\alpha]^{25}D$ +105° (*c* 2.0, 95% ethanol), separated. The filtrate was concentrated *in vacuo* to a glass. The glass was taken up in 1 l. of chloroform and extracted with 2 l. of 5% sodium hydroxide. Addition of the aqueous layer to excess hydrochloric acid yielded 124 g. (0.405 mole) of partially (*ca.* 65%) resolved (R)-warfarin, $[\alpha]^{24}D$ + 95 ± 2° (*c* 1.0, 0.5 *N* sodium hydroxide). The warfarin was dissolved in a boiling solution of 132.5 g. (0.405 mole) of quinine in 850 ml. of absolute ethanol. The solution was cooled to room temperature and 3350 ml. of dry ether was added. The solution then was held at −10° for 24 hr. Quinine-warfarin salt (201 g.) was collected by filtration in a cold room, $[\alpha]^{23}D$ −72.0 ±0.3° (*c* 2.5, 95% ethanol). This product was recrystallized twice by dissolving it in 3 ml. per g. of hot absolute ethanol, adding 12 ml. per g. of dry ether and cooling to −10°. A final recrystallization from 2.5 ml. of absolute ethanol per g. of salt (cooling to room temperature) yielded 88 g. of pure quinine-(R)-warfarin salt, $[\alpha]^{23}D$ −71.0 ± 0.3° (*c* 1.7, 95% ethanol). This salt was partitioned between 1 l. of chloroform and 1 l. of 5% sodium hydroxide and the warfarin was precipitated by the addition of the aqueous phase to excess hydrochloric acid. One crystallization from acetone–water yielded 37 g. of (R)-warfarin, m.p. 170–171°, $[\alpha]^{25}D$ +149.0 ± 0.5° (*c* 2, 0.5 *N* sodium hydroxide), +24.8 ± 1° (*c* 2, acetic acid), +15.5 ± 1° (*c* 3, acetonitrile), −16.6 ± 1° (*c* 3, dioxane), −14.8 ±1° (*c* 3.7, butanone-2) and −20.1 ± 1° (*c* 2, propanol-2).

Large prismatic crystals of the pure enantiomers were obtained by slow crystallization from acetone or acetic acid. The solubilities of the pure enantiomers at 25° were 112 mg./ml. of acetone and 26 mg./ml. of acetic acid. The solubilities of *rac*-warfarin were 65 mg./ml. of acetone and 20 mg./ml. of acetic acid. The infrared spectra of optically pure and *rac*-warfarin were identical; $\lambda_{max}^{chloroform}$ 2.78(w), 5.88, 6.16 and 6.38 μ.

(−)(S)-3-(α-Phenyl-γ-ethylenedithiobutyl)-2-hydroxychromone [(−)(S)-II].[18]—(S)-Warfarin (4 g.) was dissolved in a mixture of 8 ml. of ethanedithiol and 15 ml. of boron trifluoride ethyl ether. After 1 hr. the solution was poured into 500 ml. of ether and extracted twice with water. Extraction of the ether with 2 portions of 0.2 *N* sodium hydroxide and acidification of the extracts yielded the solid thioketal. Crystallization from 30 ml. of 95% ethanol yielded 3.5 g. of product, m.p. 193–195°, $[\alpha]^{25}D$ −127.5 ± 0.5° (*c* 1.3, 95% ethanol); λ_{max}^{KBr} 3.15, 6.06 (4-C=O) and 6.20 μ.

Anal. Calcd. for $C_{21}H_{20}S_2O_3$: C, 65.6; H, 5.2. Found: C, 65.1; H, 5.4.

(−)(S)-3-(α-Phenylbutyl)-4-hydroxycoumarin [(−)(S)-III]. **A. From the Thioketal.**—The thioketal prepared above (2.5 g.) was dissolved in 200 ml. of absolute ethanol and about 30 g. of W-7 Raney nickel was added. The slurry was stirred and refluxed 1 hr., after which it was centrifuged and the supernatant was concentrated to an oil. The oil was dissolved in aqueous base, extracted with ether, and the basic solution was added to excess hydrochloric acid to precipitate a solid product. Crystallization from alcohol–water gave 1 g. of (−)(S)-III, m.p. 132–133°, $[\alpha]^{25}D$ −110 ± 1° (*c* 2, 95% ethanol) and −162 ± 1° (*c* 1.2, 1 *N* sodium hydroxide); $\lambda_{max}^{chloroform}$ 2.95, 3.41, 5.92 (2-C=O) and 6.14 μ.

B. By Partial Resolution.—*rac*-3-(α-Phenylbutyl)-4-hydroxycoumarin[19] (200 g., 0.68 mole) and 225 g. (0.68 mole) of quinidine were dissolved in 6 l. of absolute ethanol. Filtration after 24 hr. at room temperature yielded 270 g. of salt, $[\alpha]^{23}D$ +97.8° (*c* 1.9, butanone-2). Recrystallization of this salt from 2 l. of absolute ethanol yielded 110 g. of salt, $[\alpha]^{25}D$ +83° (*c* 2, butanone-2). This salt was partitioned between chloroform and a slight excess of potassium hydroxide solution. The basic solution was added to excess hydrochloric acid and the precipitated solid was separated and dried. Crystallization from 450 ml. of absolute ethanol yielded a first crop of 17 g. of partially resolved (*ca.* 15%) (−)(S)-III, m.p. 180–200°, $[\alpha]^{25}D$ −26° (*c* 1.2, 1 *N* sodium hydroxide). Warm water (300 ml.) was added to the warmed filtrate. Cooling caused crystallization of 42 g. of partially resolved (*ca.* 85%) (−)(S)-III, m.p. 135–165°, $[\alpha]^{25}D$ −96 ± 1° (*c* 2, 95% ethanol) and −142 ± 1° (*c* 1.2, 1 *N* sodium hydroxide). The infrared spectrum was identical with that of the product made by method A, $\lambda_{max}^{chloroform}$ 2.95, 3.41, 5.92 (2-C=O) and 6.14 μ.

(−)(R)-o-Hydroxy-β-phenylcaprophenone [(−)(R)-IV].—(−)(S)-3-(α-Phenylbutyl)-4-hydroxycoumarin [(−)(S)-III], $[\alpha]^{25}D$ −142° (*c* 1.2, 1 *N* sodium hydroxide), 30 g., was refluxed 72 hr. in 200 ml. of 0.5 *N* aqueous sodium hydroxide and the reaction mixture was extracted with benzene. Vacuum distillation yielded 19 g. of (−)(R)-IV, b.p. 226–228° (14 mm.), $\alpha^{24}D$ −56.1° (neat); $\lambda_{max}^{carbon\ disulfide}$ 3.18, 3.45 (w) and 6.18 μ. In dry pyridine, (−)(R) − IV gave a violet ferric chloride test.

Anal. Calcd. for $C_{18}H_{20}O_2$: C, 80.6; H, 7.51. Found: C, 80.6; H, 7.77.

The 2,4-dinitrophenylhydrazone of (−)(R)-IV, crystallized from benzene–hexane, had m.p. 122–126°.

Anal. Calcd. for $C_{24}H_{24}N_4O_5$: C, 64.2; H, 5.40. Found: C, 64.0; H, 5.56.

(+)(R)-o-Methoxy-β-phenylcaprophenone [(+)(R)-V].—(−)(R)-o-Hydroxy-β-phenylcaprophenone (7.5 g.) was dissolved in 25 ml. of dioxane and 10 ml. of 10% aqueous sodium hydroxide. Dimethyl sulfate (20 ml.) was added with mechanical stirring. After 10 hr. the reaction mixture was extracted with 50 ml. of benzene. Removal of the benzene left an oil which crystallized from 30 ml. of heptane at −10° to yield 7.7 g. of (+)(R)-V, m.p. 73.5–75°, $[\alpha]^{23}D$ +14.3 ± 0.2° (*c* 1.2, benzene), $\lambda_{max}^{carbon\ disulfide}$ 3.20 and 6.00 μ.

Anal. Calcd. for $C_{19}H_{22}O_2$: C, 80.8; H, 7.86. Found: C, 80.5; H, 7.79.

(−)(R)-1,1-Di-(o-anisyl)-3-phenylhexane-1-ol [(−)(R)-VI].—(+)(R)-o-Methoxy-β-phenylcaprophenone (3 g.) dissolved in 100 ml. of ether was added slowly to a cooled solution of *o*-anisylmagnesium bromide made from 7 g. of *o*-bromoanisole and 4 g. of magnesium in 100 ml. of ether. After 4 hr. refluxing, 50 ml. of water and 5 ml. of acetic acid were added and the ether layer was separated, dried (magnesium sulfate) and evaporated. The residue was crystallized from 25 ml. of heptane and recrystallized from benzene–heptane to yield 2.3 g. of (−)(R)-VI, m.p. 93–95°, $[\alpha]^{22}D$ −4.1 ±0.3° (*c* 2, benzene).

Anal. Calcd. for $C_{26}H_{20}O_3$: C, 80.0; H, 7.73. Found: C, 80.0; H, 7.71.

(+)(S)-Methyl β-Phenylcaproate [(+)(S)-IX].—*rac*-β-Phenylcaproic acid (IX) was prepared and resolved by the methods of Levene and Marker.[20] Excess diazomethane in ether was added to 9.8 g. of (+)(S)-β-phenylcaproic acid

(18) We have assigned the chromone structure to II because of the carbonyl absorption at 6.06 μ. *Cf.* R. A. Abramovitch and J. R. Gear, *Can. J. Chem.*, **36**, 1501 (1958), and C. F. Spencer, J. O. Rodin, E. Walton, F. W. Holly and K. Folkers, *J. Am. Chem. Soc.*, **80**, 110 (1958).

(19) C. H. Schroeder, E. D. Titus and K. P. Link, *ibid.*, **79**, 329 (1957).

(20) P. A. Levene and R. E. Marker, *J. Biol. Chem.*, **93**, 765 (1931).

[(+)(S)-VIII], $\alpha^{24}D$ +20.4 ± 0.5° (neat)[21] in ether solution. Distillation yielded 10.0 g. of (+)(S)-methyl β-phenylcaproate, b.p. 139–142° (12 mm.), $\alpha^{24}D$ + 13.40 ± 0.05° (neat).

Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.7; H, 8.74. Found: C, 75.7; H, 8.92.

rac- and (+)(S)-1,1-Di-(*o*-anisyl)-3-phenylhexan-1-ol [*rac*-VI] and [(+)(S)-VI].—(+)(S)-Methyl β-phenylcaproate ($\alpha^{24}D$ +13.4°, neat), 3.6 g., was added dropwise to the reagent prepared from 10 g. of *o*-bromoanisole and 5 g. of magnesium. The slurry resulting was refluxed 6 hr. and dissolved by adding 100 ml. of water and 10 ml. of acetic acid. The ether layer was separated, dried (magnesium sulfate) and evaporated. The residue crystallized from 15 ml. of benzene and 75 ml. of heptane, yielding a first crop of *rac*-VI which after recrystallization from benzene–heptane weighed 2.6 g., m.p. 98–99°, $[\alpha]^{23}D$ ±0.1° (*c* 2, benzene).

Anal. Calcd. for $C_{26}H_{30}O_3$: C, 80.0; H, 7.73. Found: C, 79.7; H, 7.48.

Evaporation of the filtrate from the first crystallization of the *rac*-product yielded an oil which crystallized from 50 ml. of heptane and was recrystallized twice from ether–pentane to yield 0.75 g. of (+)(S)-VI, m.p. 93–94°, $[\alpha]^{23}D$ +4.0 ± 0.3° (*c* 2.4, benzene). The infrared spectra of the *rac.*, (+)- and (−)-alcohols were identical: $\lambda_{max}^{chloroform}$ 2.85, 3.40 and 6.23 μ.

Anal. Calcd. as above. Found: C, 80.4; H, 7.96.

(21) This rotation is higher than that reported by Levene and Marker[20] ($\alpha^{25}D$ +6.08°, neat); however, the acid is not optically pure.

[CONTRIBUTION FROM FACULTY OF PHARMACY, SCHOOL OF MEDICINE AND RESEARCH INSTITUTE FOR CATALYSIS AND DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, HOKKAIDO UNIVERSITY, SAPPORO, JAPAN]

Interaction between Synthetic ATP Analogs and Actomyosin Systems[1]

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RECEIVED SEPTEMBER 19, 1960

The following compounds were synthesized chemically as analogs of adenosine triphosphate (ATP); 6-methylamino-9-β-D-ribofuranosylpurine 5′-triphosphate (VIIIa), 6-dimethylamino-9-β-D-ribofuranosylpurine 5′-triphosphate (VIIIb), 3-β-D-ribofuranosyl-2-oxo-2,3-dihydropyrimidine 5′-triphosphate (X), 9-(4′-hydroxybutyl)-6-aminopurine 4′-triphosphate (VIb), 9-(3′-hydroxypropyl)-6-aminopurine 3′-triphosphate (VIa), 9-(2′-hydroxyethyl)-6-aminopurine 2′-triphosphate (VIc) and adenosine 5′-sulfatopyrophosphate (IX). The reactions of these analogs and deoxy-ATP with myosin B or myofibrils were investigated. The intensity of light scattered by myosin B was decreased by the addition of these compounds, except for X and XI, to the same extent as by ATP. Compound X was not hydrolyzed by myosin B. The velocities of hydrolysis of compounds other than X were of the same order of magnitude under various conditions as that of ATP. Myofibrils contracted after the addition of deoxy-ATP, VIIIa or VIIIb but not after the addition of X, VIb, VIa, VIc or IX. The initial rapid liberation of phosphate, which was shown on the hydrolysis of ATP, was not observed when VIc was the substrate. Inhibition by excess substrate was observed only in the hydrolysis of ATP and deoxy-ATP.

Introduction

The interactions between actomyosin systems and natural analogs of adenosine triphosphate (ATP), such as inosine triphosphate (ITP), uridine triphosphate (UTP), guanosine triphosphate and cytidine triphosphate, have been studied by several investigators[3–6] and especially by Blum[7] and Hasselbach.[8] These investigations have thrown some new light on the role of purine and pyrimidine bases in the contraction of muscle models.

Methods of synthesis of organic triphosphate compounds have been developed by many investigators, especially by Todd[9,10] and Khorana[11] and their collaborators, and recently by Hasselbach[12] and Kessler.[13] Therefore, it might be expected that the roles of the three parts of the ATP molecule (adenine base, ribose and triphosphate) in muscle contraction might be revealed by synthesizing drastically modified analogs of ATP and investigating their reactions with actomyosin systems at three levels, *i.e.*, mysoin B solution at a high ionic strength, its suspension at a low ionic strength and isolated myofibrils. However, to the authors' knowledge, only two reports have been published in this field. They concerned the use of diacetyl-ATP[8] and adenyl methylenediphosphonate[14] as modified compounds of ATP.

The present authors have synthesized the following ATP analogs; 6-methylamino-9-β-D-ribofuranosylpurine 5′-triphosphate (VIIIa), 6-dimethylamino-9-β-D-ribofuranosylpurine 5′-triphosphate (VIIIb), 3-β-D-ribofuranosyl-2-oxo-2,3-dihydropyrimidine 5′-triphosphate (X), 9-(4′-hydroxybutyl)-6-aminopurine 4′-triphosphate (VIb), 9-(3′-hydroxypropyl)-6-aminopurine 3′-triphosphate (VIa), 9-(2′-hydroxyethyl)-6-aminopurine 2′-triphosphate (VIc) and adenosine 5′-sulfatopyrophosphate (IX). It is the purpose of this report to describe the methods of synthesis of these analogs and the properties of their reactions with actomyosin systems and also to clarify the role of the ATP molecule in muscle contraction.

Results

The velocity of liberation of phosphate and the decrease in light-scattering by myosin B in the presence of ATP or its analog fluctuated considerably

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