chloride gas was passed into the reaction mixture at room temperature for six hours. The latter was then tightly stoppered and allowed to stand for ten days. The liquid in this time had become quite viscous and had turned deep red. The mixture was then subjected to steam distillation to free it from unreacted phenol and solvent; the non-volatile residue, which was obtained in the form of a cake, was crushed, dried, and recrystallized from benzene in the form of orange-colored plates. The crystals are soluble in alkali, m. p. 212° (uncor.).

Anal. Calcd. for $C_{26}H_{20}O_3$: C, 82.10; H, 5.26; mol. wt., 380. Found: C, 82.13; H, 5.26; mol. wt., 370.

Acetate (IIa).—Two grams of the condensation product was refluxed for two hours with 25 cc. of acetic anhydride in an all-glass apparatus. The solution was poured into cold water and was allowed to stand, with occasional shaking, till the excess reagent had hydrolyzed. The resulting semi-solid was transferred onto a porous tile till it had completely solidified, then recrystallized from alcohol, m. p. 168° (uncor.).

Anal. Calcd. for $C_{30}H_{24}O_5$: C, 77.58; H, 5.17; mol. wt., 464. Found: C, 77.30; H, 4.91; mol. wt., 461.

Propionate (IIb).—A mixture of two grams of the benzil–phenol condensation product and 25 cc. of propionic anhydride was refluxed for two hours, then poured into cold water and allowed to stand overnight. The resulting solid was recrystallized from alcohol, m. p. 123–125° (uncor.).

Anal. Calcd. for $C_{32}H_{28}O_5$: C, 78.05; H, 5.69. Found: C, 77.90; H, 5.78.

Benzhydryl-4,4'-dihydroxy-tritan (III).—Five grams of the benzil–phenol condensation product was dissolved in 50 cc. of absolute amyl alcohol in a 200 cc. round-bottomed flask provided with a reflux condenser. The solution was heated to boiling and, in the course of a half hour, five grams of freshly cut sodium was added, the mixture being gently refluxed the while. After all the sodium had been added, the solution was allowed to cool, then transferred to a separatory funnel, to which was also added 50 cc. of water and 25 cc. of ether. The mixture was thoroughly shaken and the lower aqueous layer was drawn off. The ether layer was washed with water and the washings added to the main extract, which was then acidified with dilute hydrochloric acid. The acidified mixture was then extracted with 25-cc. portions of ether; the ether extract was evaporated to dryness and the solid obtained was crystallized from benzene. The product is soluble in alkali, m. p. 152–154° (uncor.).

Anal. Calcd. for $C_{26}H_{22}O_3$: C, 81.67; H, 5.78. Found: C, 81.80; H, 5.49.

Acetate (IIIa).—The acetate of the reduced phenol was prepared in the manner previously described. The triacetate was recrystallized from alcohol, m. p. 114–116° (uncor.).

Anal. Calcd. for $C_{32}H_{28}O_6$: C, 75.60; H, 5.52. Found: C, 76.16; H, 5.40.

Summary

This communication concludes the systematic investigation of the condensations of diketones with phenols. In the course of this research tetraphenols, indano-indanes, coumarano-coumarans, tritan, naphthalene and anthracene compounds were produced.

WASHINGTON SQUARE COLLEGE
NEW YORK, N. Y. RECEIVED JANUARY 20, 1941

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

The Resolution of Racemic Pantothenic Acid by Means of Quinine Methohydroxide

BY ERIC T. STILLER AND PAUL F. WILEY

The syntheses of *dextro-* and *levo*-rotatory pantothenic acid from the optical enantiomorphs of α,γ-dihydroxy-β,β-dimethylbutyric acid have already been described by Stiller, *et al.*[1] In this same paper the preparation of racemic pantothenic acid was also described. In the interim, the resolution of racemic pantothenic acid has been described by Kuhn and Wieland[2] who made use of the corresponding quinine salt.

The present paper deals with the optical resolution of the racemic pantothenic acid by means of a novel reagent.[3]

Preliminary experiments indicated that some of the alkaloidal salts of *dl*-pantothenic acid did not lend themselves to continued recrystallization owing to decomposition. A stronger optically active base was sought which would form a readily crystallizable salt with the racemic acid. Attention was, therefore, turned to the optically active quaternary ammonium hydroxides.

The salt obtained by the neutralization of an aqueous solution of racemic pantothenic acid with an aqueous solution of quinine methohydroxide was crystallized readily from an alcohol–ether mixture.

The least soluble fraction of the quaternary ammonium salt gave, after six recrystallizations, the pure salt of (+)-pantothenic acid ($(\alpha)^{25}D$ −122.0°; m. p. 197°), in admixture with a

(1) Stiller, Harris, Finkelstein, Keresztesy and Folkers, THIS JOURNAL, **62**, 1785 (1940).
(2) Kuhn, Wieland, *Ber.*, **73**, 971 (1940).
(3) Major and Finkelstein, THIS JOURNAL, **63**, 1368 (1941).

sample of quinine methopantothenate ($(\alpha)^{25}D$ −118°) prepared from calcium (+)-pantothenate, it showed no depression of the m. p. Further, both samples of quinine methopantothenate showed 100% activity on bacterial assay.

Further fractionation of the original mother liquor gave two small fractions of intermediate composition and bacterial activity, and finally a third fraction which had a very low bacterial activity. This fraction after several crystallizations from alcohol–ether gave a product which had a bacterial activity of 0.1%; $(\alpha)^{25}D$ −156.5°, m. p. 170°. The mixed m. p. with a specimen prepared from calcium (−)-pantothenate, showed no depression.

Final confirmation of the identity of the optical enantiomorphs was obtained by the decomposition of the alkaloidal salts followed by the conversion of the resulting (+)- and (−)-pantothenic acids into their *s*-benzylthiuronium salts. These were identical with specimens prepared from (+)- and (−)-pantothenic acids obtained from the enantiomorphs of the resolved α-hydroxy-β,β-dimethyl-γ-butyrolactone.

The carbon analyses of all of the samples of the quinine metho salts, both resolved and authentic, ran consistently low (0.5–0.6%). A second resolution was, therefore, carried out using quinine methohydroxide which had been prepared from rigorously purified quinine. The analytical results, however, again gave low carbon values.

The racemic pantothenic acid was also resolved by means of its cinchonidine salt. After three recrystallizations from an alcohol–ether mixture, the pure cinchonidine salt of (+)-pantothenic acid was obtained ($(\alpha)^{28.4}D$ −60.55°; m. p. 176–177°). It gave no depression of m. p. in admixture with an authentic sample of the cinchonidine salt prepared from (+)-pantothenic acid. On bacterial assay it showed 102% activity.

Experimental Part

Resolution of *d,l*-Pantothenic Acid.—An aqueous solution of 32 g. of *d,l*-pantothenic acid in 50 cc. of water was cooled and neutralized to *p*H 7.5 with an aqueous solution of quinine methohydroxide, prepared by stirring quinine methochloride in aqueous suspension with silver oxide. After standing for some hours at 0° a small amount of crystalline material (0.27 g.; m. p. 196°) was obtained which had no effect on the growth stimulation of *Lactobacillus casei*. The aqueous solution was concentrated to half its volume *in vacuo* below 25°, and a further small quantity of bacteriologically inactive material was removed (0.7 g.). The solution was then evaporated to dryness below 25° and the residue was dried by dissolving in absolute alcohol (20 cc.), adding 75 cc. of benzene and removing the solvents by distillation *in vacuo*. The residue was finally dried in a desiccator over sulfuric acid.

The dry, partially crystalline residue was ground to a fine powder with 250 cc. of ethyl acetate in order to remove a small amount of gummy material which hindered crystallization of the product; yield, 65.6 g. This material had m. p. 177°; $(\alpha)^{27}D$ −132.1° (*c*, 0.71% in MeOH); bacterial assay 40%.

By fractional crystallization of the product from 100 cc. of alcohol by the successive addition of 100-cc. portions of ether, four crops of crystals were obtained as shown in Table I.

TABLE I

Fraction	M. p., °C.	$[\alpha]D$	Weight, g.	Bacterial assay, %
A	182–184	−129°	22.6	46.4
B	177	−132.2°	3.7	51.0
C	177	−127.8°	0.5	36.6
D	163	−150.0°	6.3	5.4

After six recrystallizations of Fraction A from the alcohol–ether mixture (1:2) 4.8 g. clusters of small, fine, colorless needles was obtained which showed 100% activity on bacterial assay. It also showed $(\alpha)^{25}D$ −122.0°, (*c*, 4.17% in MeOH); m. p. 196–197° with material prepared from (+)-pantothenic acid.

Anal. Calcd. for $C_{30}H_{43}O_7N_3$: C, 64.63; H, 7.72; N, 7.54. Found: C, 64.12; H, 7.90, 8.05; N, 7.48, 7.43, 7.49.

Fraction D was recrystallized five times from alcohol–ether (1:2) and was then obtained in clusters of fine colorless needles, m. p. 170°, mixed m. p. with a sample prepared from (−)-pantothenic acid, 170°. It showed $(\alpha)^{25}D$ −156.5° (*C*, 0.96% in MeOH); and bacterial assay 0.1%; yield 4.1 g.

Anal. Calcd. for $C_{30}H_{43}O_7N_3$: C, 64.63; H, 7.72; N, 7.54. Found: C, 63.97; H, 7.97; N, 7.53.

Quinine Metho Salt of (+)-Pantothenic Acid.—A solution of 0.9814 g. of calcium (+)-pantothenate in 4 cc. of water was treated with an equivalent amount of *N* sulfuric acid with cooling. The calcium sulfate was removed by filtration and washed with a small quantity of ice-water. The combined filtrates were adjusted to *p*H 5.5 with pyridine and evaporated to dryness *in vacuo*, at 25°. The residue after drying in high vacuum was extracted with 20 cc. of acetone and the insoluble calcium sulfate removed by filtration. On removal of the acetone by evaporation *in vacuo*, the pure (+)-pantothenic acid was obtained as a colorless viscous oil; yield 0.8967 g.

The product was dissolved in 5 cc. of water and neutralized to *p*H 7.5 with an aqueous solution of quinine methohydroxide. After standing for some hours at 0° the solution was evaporated to dryness at 25° *in vacuo*, thoroughly dried in high vacuum, and then triturated with ethyl acetate. The crystalline product was recrystallized from alcohol–ether (1:2) and obtained as clusters of fine colorless needles; m. p. 196–197°; $(\alpha)D$ −118.5° (*C*, 1.20% in MeOH); bacterial assay 101; 99.7%; yield 1.39 g.

Anal. Calcd. for $C_{30}H_{48}O_7N_2$: C, 64.63; H, 7.72; N, 7.54. Found: C, 64.01, 64.08, 64.10; H, 7.92, 7.72, 7.63; N, 7.41, 7.52.

Quinine Metho-(−)-pantothenate.—The salt was prepared as described above for the (+)-salt. The product was obtained as clusters of fine colorless needles and showed m. p. 170°; $(\alpha)^{25}D$ −156.0° (*C*, 0.80% in MeOH); bacterial assay, negative.

Conversion of the Quinine Metho Salts of (+)- and (−)-Pantothenic Acid into the Corresponding *s*-Benzylthiuronium Salts.—The quinine metho salts were dissolved in a small amount of water and the theoretical amount of *N* sulfuric acid added to the cold solution. The precipitate of quinine methosulfate was removed by filtration. The filtrate was then adjusted to *p*H 5.5 with pyridine and the water removed *in vacuo* at 25°. The dried residue was extracted with acetone and filtered from a small amount of insoluble material and the acetone removed under reduced pressure. The *s*-benzylthiuronium salt was prepared from a solution of the neutral sodium salt of the pantothenic acid in methanol by the addition of 1.1 moles of *s*-benzylthiuronium chloride and the solution allowed to stand for several hours. After evaporation of the solvent, the salt was separated from sodium chloride by extraction with boiling acetone.

After recrystallization from acetone both the (+) and (−) salts were obtained as fine colorless needles.

***s*-Benzylthiuronium of Resolved (+)-Pantothenate.**—M. p. 148–149°, mixed m. p. with an authentic sample 149°.

Anal. Calcd. for $C_{17}H_{27}O_5N_3S$: C, 52.95; H, 7.07; N, 10.90. Found: C, 52.92; H, 6.87; N, 10.93.

***s*-Benzylthiuronium of Resolved (−)-Pantothenate.**—M. p. 150–151°, mixed m. p. with an authentic sample 151°.

Anal. Calcd. for $C_{17}H_{27}O_5N_3S$: C, 52.95; H, 7.07; N, 10.90. Found: C, 53.03; H, 7.12; N, 10.84.

The mixed m. p. of the (+) and (−) salts was 138°.

***s*-Benzylthiuronium Salt of (+)-Pantothenic Acid from Calcium (+)-Pantothenate.**—The salt was obtained from calcium (+)-pantothenate by conversion into the sodium salt followed by treatment with *s*-benzylthiuronium chloride. The product was isolated as described above. It has m. p. 149°.

Anal. Calcd. for $C_{17}H_{27}O_5N_3S$: C, 52.95; H, 7.07; N, 10.90. Found: C, 52.92; H, 6.87; N, 10.93.

***s*-Benzylthiuronium Salt of (−)-Pantothenic Acid from Calcium (−)-Pantothenate.**—This salt was prepared as described above from calcium (−)-pantothenate. After recrystallization from acetone or isopropanol it was obtained as thin colorless scales, m. p. 151°.

Anal. Calcd. for $C_{17}H_{27}O_5N_3S$: C, 52.95; H, 7.07; N, 10.90. Found: C, 52.84; H, 7.32; N, 11.07.

The Resolution of *d,l*-Pantothenic Acid by Means of the Cinchonidine Salt.—To a solution of 27 g. of *d,l*-pantothenic acid in 100 cc. of acetone, 36 g. of cinchonidine was added and the mixture boiled under reflux. Methyl alcohol was added until complete solution of the cinchonidine was obtained (140 cc.), and the refluxing continued for thirty minutes. On cooling, crystallization did not take place and the solvents were removed by distillation at 25° *in vacuo*. The residue was dissolved in 100 cc. of alcohol and 50 cc. of ether added. On standing at 0°, colorless fluffy needles were deposited (20 g.). After two recrystallizations from alcohol–ether, 13 g. of colorless needles was obtained, m. p. 176–177°, which showed no depression of the melting point when mixed with an authentic sample It showed $(\alpha)^{28.4}D$ −60.6° (*C*, 1.35% in MeOH) and bacterial assays showed 101–102% activity.

Anal. Calcd. for $C_{28}H_{39}O_6N_3$: C, 65.49; H, 7.65; N, 8.18. Found: C, 65.55; H, 7.78; N, 8.11.

By the addition of further quantities of ether to the original mother liquor, and recrystallization of the product, a further 7 g. of the pure cinchonidine salt of (+)-pantothenic acid was obtained.

It was not found possible to isolate the cinchonidine salt of (−)-pantothenic acid. By fractional crystallization of the mother liquors, a fraction was obtained with some difficulty showing a bacterial activity of 19.6% (m. p. 143–145°). This salt was apparently somewhat unstable and crystallized with difficulty. During a subsequent recrystallization, it partially decomposed and the pantothenic acid was, therefore, recovered by the usual methods and showed a bacterial activity of 10.3%.

Cinchonidine Salt of (+)-Pantothenic Acid.—The cinchonidine salt of (+)-pantothenic acid was prepared from 0.5 g. (+)-pantothenic acid as described above. It was obtained as colorless needles, m. p. 177–178°. It had $(\alpha)^{25}D$ −61.3° (*C*, 0.67% in MeOH); bacterial assay, 101%.

Anal. Calcd. for $C_{28}H_{39}O_6N_3$: C, 65.49; H, 7.65; N, 8.18. Found: C, 65.31; H, 7.60; N, 8.28.

Acknowledgment.—The authors wish to express their thanks to Drs. Randolph T. Major and Karl Folkers for their interest and council; to Messrs. D. F. Hayman, W. Reiss and H. S. Clark for carrying out the microanalyses; to Mr. M. Kasha for carrying out the bioassays; and to Mr. W. B. Wright for his assistance throughout the investigation.

Summary

1. *d,l*-Pantothenic acid has been resolved by means of its quinine metho salt.

2. The resulting quinine metho salts of (+)- and (−)-pantothenic acids were identical with specimens prepared from the pure acids. The enantiomorphic acids were also compared as their *s*-benzylthiuronium salts with authentic samples.

3. *d,l*-Pantothenic acid has also been resolved by means of its cinchonidine salt. The (+)-salt was identical with a specimen prepared from calcium (+)-pantothenate. The (−)-salt was not isolated.

4. The (+)-salts from both resolutions showed full growth stimulation activity when assayed with *Lactobacillus casei*. The (−)-salts had practically no activity when assayed by the same method.

RAHWAY, N. J. RECEIVED FEBRUARY 11, 1941