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A Novel Resolution of Pantolactone—The Synthesis of D(+)-Calcium Pantothenate[1]

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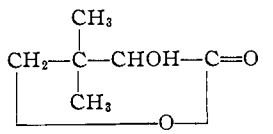
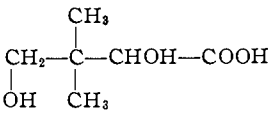
D(−)-Galactamine, a novel resolving agent, was used to resolve pantolactone, a key intermediate in the synthesis of D(+)-calcium pantothenate. Pantolactone reacted with D(−)-galactamine to yield a pair of diastereoisomeric N-D-dulcityl-2,4-dihydroxy-3,3-dimethylbutyramides. Fractional crystallization yielded (+)-N-D-dulcityl-D-2,4-dihydroxy-3,3-dimethylbutyramide in 70% yield (98% optical purity) and the (−)-diastereoisomer in 80% yield (93–98% optical purity). D(−)-Pantolactone and D(−)-galactamine were recovered from the (+)-diastereoisomer in 94 and 96% yields, respectively, by acid hydrolysis. The calcium salt of β-alanine was coupled with D(−)-pantolactone in methyl Cellosolve at 25–30° to yield crystalline D(+)-calcium pantothenate in 94% yield based on pantolactone.

Pantolactone (I), a key intermediate in the synthesis of pantothenic acid, previously has been resolved by conversion to diastereoisomeric salts of the corresponding 2,4-dihydroxy acid (II)[2–6] by



I



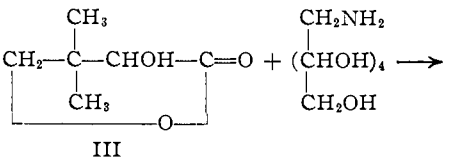
II

conversion of the secondary hydroxyl group to a half-ester followed by salt formation[7–9] and through the formation of molecular complexes with brucine.[7]

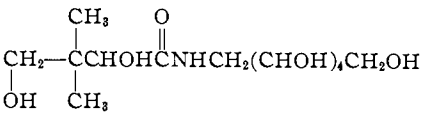
With the exception of the pyridinium diacetyl tartrate resolution,[7] each of the above schemes utilized a relatively expensive alkaloid[2–4,7–9] or a synthetic material which itself had to be resolved before use.[5,6] Our initial objective was to find an optically active base useful in the resolution of pantolactone and sufficiently inexpensive so that its near quantitative recovery would not be a critical factor in the successful operation of a commercial process. To achieve such an objective, one must necessarily prepare the resolving agent from inexpensive, naturally occurring, optically active materials. Since certain sugars are the least expensive of the pure optically active organic compounds available to the chemist, it seemed desirable to use a sugar or one of its derivatives for resolution work. The benzimidazole base formed by D-*gluco*-D-*gulo*-heptonolactone and *o*-phenylenediamine has been used successfully to resolve tartaric acid[10]; however, no report has yet appeared on the use of a simple basic sugar derivative such as a glycamine as a resolving agent. Since the glycamines appeared to meet our requirements for a readily available, inexpensive resolving agent, we undertook a program aimed at determining their applicability to the resolution of pantolactone.

Glucamine, potentially the least expensive of the glycamines, was prepared by the hydrogenolysis of N-benzylglucamine.[11] Galactamine was prepared by the method of Flint and Salzberg[12] as modified by Holly and co-workers.[13] The crude product obtained by the latter method was not suitable for resolution work. It was purified through the salicylidene or benzylidene derivatives to yield a material which was essentially pure galactamine.[11]

Glucamine failed to form crystalline derivatives with pantolactone; however, galactamine condensed smoothly with the lactone to form crystalline diastereoisomeric forms of N-D-dulcityl-2,4-dihydroxy-3,3-dimethylbutyramide (IV), hereafter referred to as dulcitylpantamide. The reaction occurred readily in refluxing ethanol or by fusion at 100–125°. N-Substituted glycamines such as N-methylglucamine and N-benzylglucamine failed to react with pantolactone in refluxing ethanol, refluxing pyridine or by fusion.



III



IVa, dextro, m.p. 117–118°
b, levo, m.p. 122–123°

In contrast with glucamine, galactamine proved to be a very satisfactory resolving agent. Early experiments in which this agent was used to resolve pantolactone yielded dextrorotatory dulcitylpantamide (IVa), m.p. 117–118°, and the levo diastereoisomer IVb, m.p. 122–123°. Solubility studies in ethanol at 25° indicated that the dextro diastereoisomer was about 30% more soluble than the levo form. During resolution, the first compound to crystallize from the reaction mixture was the unexpected, more soluble dextro compound. As the initial dextro enriched crops crystallized from solution, the composition of the mother liquor became

(1) Presented in part at the 129th Meeting of The American Chemical Society, Dallas, Texas, April, 1956.

(2) S. A. Harris and K. Folkers, U. S. Patent 2,319,545 (May 18, 1943); *C. A.*, **37**, 6280 (1943).

(3) R. H. Beutel and M. Tishler, U. S. Patent 2,474,719 (June 28, 1949); *C. A.*, **43**, 7038 (1949).

(4) R. T. Major and J. Finkelstein, THIS JOURNAL, **63**, 1368 (1941).

(5) F. D. Pickel, J. I. Fass and S. Chodroff, U. S. Patent 2,460,239 (Jan. 25, 1949); *C. A.*, **43**, 3448 (1949).

(6) F. D. Pickel and S. Chodroff, U. S. Patent 2,460,240 (Jan. 25, 1949); *C. A.*, **43**, 3448 (1949).

(7) R. Beutel and M. Tishler, THIS JOURNAL, **68**, 1463 (1946).

(8) J. Finkelstein, U. S. Patent 2,328,000 (Aug. 31, 1943); *C. A.*, **38**, 837 (1944).

(9) F. Bergel, A. Cohen, A. L. Morrison and A. R. Moss, U. S. Patent 2,423,062 (June 24, 1947); *C. A.*, **41**, 6278 (1947).

(10) W. T. Haskins and C. S. Hudson, THIS JOURNAL, **61**, 1266 (1939).

(11) F. Kagan, M. A. Rebenstorf and R. V. Heinzelman, *ibid.*, **79**, 3541 (1957).

(12) R. B. Flint and P. L. Salzberg, U. S. Patent 2,016,962 (Oct. 8, 1935); *C. A.*, **29**, 8007 (1935).

(13) F. W. Holly, E. W. Peel, R. Mozingo and K. Folkers, THIS JOURNAL, **72**, 5416 (1950).

predominantly levo until a 5–10% enrichment was reached. At this point, levo isomer was deposited until the composition of the mother liquor again approached that of a racemate. This behavior was then repeated in a cyclic fashion. Since an appreciable displacement of the composition of the mother liquor from that of a racemate was impossible, and, furthermore, since optical purity appeared to be inversely related to the size of the crops which were isolated, this method of resolution of pantolactone was tedious.

In one of the galactamine resolution experiments a new material was obtained which melted 40° higher than either of the previously isolated dulcitylpantamides (IVa and IVb). Potentiometric titration showed that the compound was not a dulcitylammonium salt of 2,4-dihydroxy-3,3-dimethylbutyric acid. Since acid hydrolysis yielded galactamine hydrochloride and L(+)-pantolactone, it appeared that the new material was a polymorphic form of *l*-dulcitylpantamide. A mixed melting point between the previously isolated *l*-dulcitylpantamide (m.p. 122–123°, IVb) and the new material (m.p. 162.5–165°, IVc) was not depressed, and the specific rotations of the two materials in water were identical. The new polymorph, synthesized from D(−)-galactamine and L(+)-pantolactone by the methods which had previously yielded the low melting material, was designated the "β"-form of (−)-dulcitylpantamide. In resolution work in these laboratories, the "α"-form (IVb) had been used for several years with no apparent instability. Samples stored at room temperature in screw-cap vials remained unchanged; however, attempts to recrystallize existing samples of "α"-(−)-dulcitylpantamide have thus far yielded only the "β"-form (IVc).

Since the "β"-form of (−)-dulcitylpantamide proved to be very insoluble in hot ethanol compared to the (+)-diastereoisomer, an excellent separation of the two materials was possible. In one resolution of 100 g. of pantolactone, an 80% yield of (−)-dulcitylpantamide was obtained in 93–98% optical purity along with a 70% yield of the D(+)-isomer in 96% optical purity. The residual material and the small intermediate fractions of lesser purity could be recycled.

(+)-Dulcitylpantamide was hydrolyzed with dilute hydrochloric acid to give a 94% yield of D-(−)-pantolactone based on the starting amide and a practically quantitative yield of galactamine hydrochloride. The latter was converted to the free base by passage of an aqueous solution through a bed of anion exchange resin. The over-all yield of recovered galactamine was 96% from (+)-dulcitylpantamide. This material was suitable for further resolution work with no additional purification.

The biologically inactive L(+)-pantolactone was easily racemized by the procedure of Stiller and co-workers,[14] by fusing with trisodium phosphate at 160° or by merely heating to 195° in the presence of soft glass. The soft-glass method is similar to that of Klein and Kapp[15] in which they used alkali metal silicates as their catalyst. Both trisodium phosphate and the soft-glass procedures convert L(+)-pantolactone to the racemic form in over 95% yield.

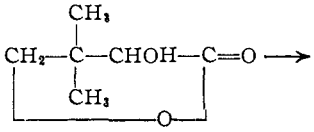
The final step in the synthesis of pantothenic acid is the coupling of β-alanine or one of its salts with pantolactone. If β-alanine is used, a temperature of about 180°[16] is necessary to effect reaction, presumably because the zwitterion form of the amino acid is relatively inactive toward attacking the carbonyl group of pantolactone. The procedures in which salts of β-alanine are used in the coupling with pantolactone are often carried out in refluxing anhydrous alcohol[17] and in aqueous solution.[18,19] These methods are usually complicated since the product, a salt of pantothenic acid, is first isolated as an amorphous hygroscopic solid by precipitation from alcoholic solution by the addition of ethyl acetate or acetone. A pure crystalline product is obtained only after crystallization from methanol.

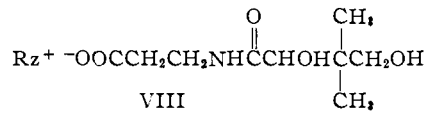
We attempted to block zwitterion formation in β-alanine and thus free the amino group for reaction with pantolactone by adsorbing the amino acid on a strongly basic anion exchange resin (IR-410) to form the resin salt VII. Coupling with pantolactone was then carried out to form the resin pantothenate (VIII). The product was then displaced from the resin by calcium chloride solution. In addition to D(+)-calcium pantothenate (15% yield), we obtained a material (49% yield) which melted at 226–230° dec., approximately 30° higher than calcium pantothenate. Microbiological assay of this material with *lactobacillus arabinosus* indicated a D(+)-calcium pantothenate activity of 67%. The microbiological assay, the analytical data and the infrared spectrum indicated that the product was a mixed salt of pantothenic acid and 2,4-dihydroxy-3,3-dimethylbutyric acid (X).

We found that the reaction of D(−)-pantolactone with the calcium salt of β-alanine proceeded

(a) $NH_2CH_2CH_2COOH + Rz^+OH^- \longrightarrow Rz^+ {}^-OOCCH_2CH_2NH_2$

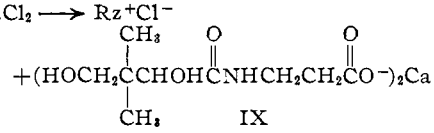
VII, Rz = Resin

(b) VII +  →



VIII

(c) VIII + $CaCl_2 \longrightarrow Rz^+Cl^-$ +



IX

(14) E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesy and K. Folkers, THIS JOURNAL, **62**, 1785 (1940).

(15) H. C. Klein and R. Kapp, U. S. Patent 2,688,027 (Aug. 31, (1954); *C. A.*, **49**, 13289 (1955).

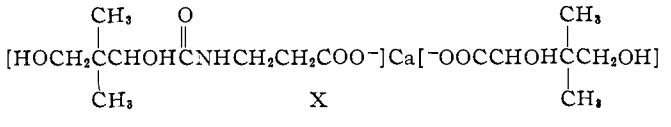
(16) M. B. Moore, U. S. Patent 2,234,680 (March 11, 1941); *C. A.*, **35**, 4041 (1941).

(17) F. D. Pickel and H. H. Weinstock, U. S. Patent 2,442,143 (May 25, 1948); *C. A.*, **42**, 5174 (1948).

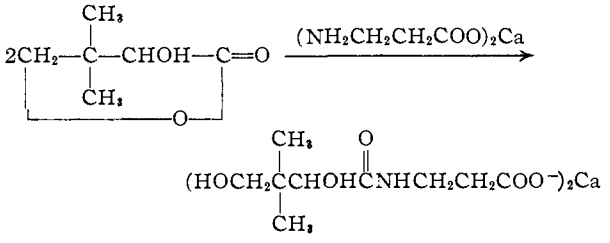
(18) S. H. Babcock, Jr., U. S. Patent 2,375,885 (May 15, 1945); *C. A.*, **39**, 3399 (1945).

(19) S. H. Babcock, Jr., U. S. Patent 2,441,949 (May 25, 1948); *C. A.*, **42**, 7788 (1948).

smoothly in methyl Cellosolve at room temperature or slightly above (35°) to yield calcium panto-



thenate in 94% yield based on the lactone.



The attractive aspect of using methyl Cellosolve as the solvent in this reaction was that the product, D(+)-calcium pantothenate, crystallized directly from the reaction mixture in excellent yield in an analytically pure condition.

cess pantolactone was removed by extraction with five 40-ml. portions of methylene chloride, and the aqueous solution was concentrated under reduced pressure to a viscous pale yellow sirup which was dried by repeated distillation of absolute ethanol. The dried residue was dissolved in 65 ml. of hot ethanol, seeded with "α"-(−)-dulcitylpantamide and stored at 3°. Five crops of crystals were removed over a period of two weeks. The pertinent data for these crops are summarized in Table I. After the removal of the fifth crop, the mother liquor was concentrated to a sirup which was dissolved in 25 ml. of water and extracted well with methylene chloride. The aqueous solution was evacuated at 15 mm. to remove residual methylene chloride and was filtered through a column of Darco–Celite (1:2) (5 cm. × 1 cm.). The golden yellow solution was decolorized effectively by this treatment. The filtrate was concentrated under reduced pressure to a viscous sirup which was dissolved in 18 ml. of hot ethanol. The solution was seeded with (+)-dulcitylpantamide and stored at 3°. After two days, a white crystalline solid, crop 6, was removed by filtration, 2.0 g. (11%), m.p. 149–152°, $[\alpha]_D$ −25°. This material melted about 30° higher than any crop previously isolated in the resolution of pantolactone with galactamine. Crop 6 was recrystallized three times from ethanol–water to yield an analytical sample of "β"-(−)-dulcitylpantamide, m.p. 162.5–165°.

TABLE I

SUMMARY OF CROPS REMOVED FROM THE RESOLUTION IN WHICH "β" (−)-DULCITYLPANTAMIDE WAS ISOLATED

Crop	1	2	3	4	5	6
Weight, g.	6.7	1.4	2.1	0.5	1.0	2.0
Theor. amides, %	35	7	11	3	5	11
M.p., °C.	107–112	100–110	111–117	115–122	92–102	149–152
$[\alpha]_D$ (°)	−2	−20	−28	−29	0	−25
Major component,[a] %	71% D	71% "α"-L	89% "α"-L	91% "α"-L	75% D	82% "β"-L

[a] (+)-Dulcitylpantamide is designated D and (−)-dulcitylpantamide L.

Experimental[20]

(+)-N-D-Dulcityl-D-2,4-dihydroxy-3,3-dimethylbutyramide (IVa).—D(−)-Galactamine (0.585 g., 3.2 millimoles) and 0.763 g. (5.9 millimoles, 81% excess) of D(−)-pantolactone were fused in a test-tube on a steam-bath for 4 hr. The hard glass which formed on cooling was pulverized and triturated with 5 ml. of absolute ethanol. The residual solid was dissolved in 12 ml. of hot ethanol, filtered and stored in a refrigerator overnight. The copious white solid which precipitated was removed by filtration, washed with ethanol and dried, yielding 0.68 g. (68%) of IVa, m.p. 115–116°. Recrystallization from hot absolute ethanol yielded 0.56 g. of (+)-N-D-dulcityl-D-2,4-dihydroxy-3,3-dimethylbutyramide, m.p. 117–118°, $[\alpha]_D$ +11°.

Anal. Calcd. for $C_{12}H_{25}NO_8$: C, 46.29; H, 8.09; N, 4.50. Found: C, 46.28; H, 8.12; N, 4.66.

(−)-N-D-Dulcityl-L-2,4-dihydroxy-3,3-dimethylbutyramide (IVb).—D(−)-Galactamine (1.4 g., 78 millimoles), 1.4 g. (10 millimoles, 34% excess) of L(+)-pantolactone and 75 ml. of absolute ethanol were heated to reflux with stirring for 12 hr. The resulting yellow reaction mixture was clarified by centrifugation and was stored at room temperature overnight. The solid which separated was removed by centrifugation. Recrystallization from ethanol yielded an analytical sample, m.p. 122–123°, $[\alpha]_D$ −33°.

Anal. Calcd. for $C_{12}H_{25}NO_8$: C, 46.29; H, 8.09; N, 4.50. Found: C, 46.63; H, 8.10; N, 4.24.

The Resolution of Pantolactone with D(−)-Galactamine. (a) Typical Resolution Involving "α"-(−)-Dulcitylpantamide (IVb). The Isolation of "β"-(−)-Dulcitylpantamide (IVc).—Pantolactone (17.4 g., 0.134 mole), 11.2 g. (0.062 mole) of D(−)-galactamine and 200 ml. of absolute ethanol were heated to reflux with vigorous stirring in a nitrogen atmosphere for 7 hr. The cloudy reaction mixture was filtered yielding a clear pale yellow filtrate (*p*H 8). The ethanol was removed under reduced pressure and the residual sirup was dissolved in 40 ml. of hot water. The ex-

Anal. Calcd. for $C_{12}H_{25}NO_8$: C, 46.29; H, 8.09; N, 4.50. Found: C, 46.46; H, 8.32; N, 4.37.

(b) Resolution Involving "β"-(−)-Dulcitylpantamide.—Pantolactone (100 g., 0.769 mole, 99% excess) was dissolved in 500 ml. of absolute ethanol, and about 100 ml. of the ethanol was removed by distillation to dry the lactone. The solution was cooled below the boiling point, D(−)-galactamine (70 g., 0.387 mole) was added and the suspension was stirred vigorously at the reflux temperature in a nitrogen atmosphere for 17.5 hr. The reaction mixture (*p*H 7) was cooled to room temperature, and the solid which was present was removed by filtration, 47.2 g. (29% of the theoretical amides), m.p. 161.5–163.5°, $[\alpha]_D$ −30°. The filtrate was concentrated under reduced pressure to a sirup which was taken up in 200 ml. of warm water. The aqueous solution was extracted six times with 125 ml. portions of methylene chloride, the combined extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to a semi-solid (50 g., 100%) recovered pantolactone. The aqueous solution was concentrated under reduced pressure to a burnt orange colored sirup which was dried by distillation of 100 ml. of absolute ethanol and dissolved in 200 ml. of hot alcohol. It was seeded with "β"-(−)-dulcitylpantamide and stored at 30° for 4 hr. The white crystalline solid which separated was removed by filtration, washed with absolute ethanol and dried in a vacuum oven at 50°, (34.1 g., 28%) of the theoretical amides, m.p. 112–115°, $[\alpha]_D$ +9°. It is surprising that practically pure (+)-dulcitylpantamide was obtained in this crop in spite of the fact that the solution was seeded with the L(−)-isomer. After removal of two intermediate crops totaling 12.5 g. (10%), an additional crop of pure D(+)-diastereoisomer was isolated, 7.5 g. (6%), m.p. 103–111°, $[\alpha]_D$ +9°. A summary of the crystalline crops isolated in this resolution is presented in Table II.

Hydrolysis of N-D-Dulcityl-2,4-dihydroxy-3,3-dimethylbutyramide. Proof of Structure of the "β"-(−)-Enriched Form.—"β"-(−)-N-D-Dulcityl-L-2,4-dihydroxy-3,3-dimethylbutyramide (6.6 g., 0.0212 mole, m.p. 160–162.5°, $[\alpha]_D$ −30°, 93% (−)-diastereoisomer) was heated on a steam-bath with 40 ml. of 6% by volume hydrochloric acid

(20) All melting points are uncorrected. Specific rotations were taken in water at 23°, at a concentration of 1–4% in a 2-decimeter tube.

TABLE II

RESOLUTION OF PANTOLACTONE WITH D(−)-GALACTAMINE INVOLVING "β"-(−)-DULCITYLPANTAMIDE

Crop							
Weight % of theor. amides	39	28	6	5	6	1	12
Cum. weight, %	39	67	73	78	84	85	97
M.p., °C.	161–163	112–115	120–135	149–153	103–111	162–164	Sirup
$[\alpha]_D$ (°)	−30	+9	−7	−23	+9	−32	.....
Major component, %	93% L	96% D	59% D[a]	77% L[a]	96% D	98% L	*ca.* 63% D[a]

[a] Can be recycled.

for 3 hr. The resulting solution was extracted with five 25-ml. portions of methylene chloride and the combined extracts were dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded 2.3 g. (83%) of pantolactone which was purified for analysis by sublimation ($[\alpha]_D$ +43°, equivalent to 93% of the L(+)-enantiomorph). From another experiment in which the starting material to be hydrolyzed melted at 148–151°, the pantolactone which was obtained melted at 86.6–90°, $[\alpha]_D$ +16° [66% L(+)].

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.45; H, 7.61.

The aqueous phase was concentrated under reduced pressure to a white crystalline solid which was further dried by distillation of absolute ethanol, reslurried in ethanol and filtered to yield galactamine hydrochloride in 97% yield, m.p. 158–161°.

Anal. Calcd. for $C_6H_{16}ClNO_5$: Cl, 16.29. Found: Cl, 16.66.

A mixed melting point with an authentic sample of galactamine hydrochloride was not depressed and the infrared spectra of the two samples were identical.

(b) **D(+)-Form.**—(+)-N-D-Dulcityl-D-2,4-dihydroxy-3,3-dimethylbutyramide (31.0 g., 99.7 millimoles) was hydrolyzed by the above procedure to yield 12.2 g. (94% yield) of D-(−)-pantolactone ($[\alpha]_D$ −48°, 98% D(−)-enantiomorph). The galactamine hydrochloride was dissolved in 500 ml. of deionized water and the resulting solution was passed through a ¾″ × 18″ column of Rohm and Haas IR-410 anion exchange resin in the hydroxide cycle at the rate of 5 ml. per minute. The column was washed with water until the *p*H of the effluent was 7.5. The aqueous solution of galactamine was then concentrated to dryness under reduced pressure in a nitrogen atmosphere. The white crystalline residue was dried by repeated distillation of absolute ethanol and finally by evacuation with an oil-pump to yield 17.3 g. of galactamine (96%), m.p. 142–146°.

Anal. Calcd. for $C_6H_{15}NO_5$: neut. equiv., 181.2. Found: neut. equiv., 188.

This material when treated with D(−)-pantolactone yielded (+)-dulcitylpantamide in 90% yield in one crop of crystals. The mother liquor was not worked up further for additional crops.

The Racemization of L(+)Pantolactone. (a) **Trisodium Phosphate Procedure.**—L(+)-Pantolactone [19.2 g., 0.148 mole, $[\alpha]_D$ +45° (H_2O)] was fused with 0.96 g. of trisodium phosphate decahydrate with stirring at 160° for 18 hr. The product was then flash-distilled to yield 18.5 g. (95.4%) of racemic pantolactone, $[\alpha]_D$ +1°.

(b) **Soft-glass Procedure.**—L(+)-Pantolactone (10 g.) was fused at 195° with 2.1 g. of powdered soft glass with stirring for 16 hr. The lactone was then flash-distilled at 15 mm. to yield 9.7 g. (97%) of racemic pantolactone, $[\alpha]_D$ 0°.

Racemization of L(−)-N-D-Dulcityl-L-2,4-dihydroxy-3,3-dimethylbutyramide.—Sodium metal (0.5 g., 0.022 gram atom) was dissolved in absolute methanol in a stainless steel autoclave with careful exclusion of moisture. L(−)-N-D-Dulcityl-2,4-dihydroxy-3,3-dimethylbutyramide (5.0 g., 0.165 mole) was added, and the reaction mixture was heated at 100° for 2 hr. The methanol was removed under reduced pressure and the residue was taken up in 125 ml. of water. The aqueous solution was passed through a bed of Rohm and Haas IR-120 cation exchange resin in the hydrogen cycle (1 cm. × 17 cm.) to remove caustic. The effluent solution was concentrated to a sirup under reduced pressure yielding 4.1 g. of residue (82%) which was dissolved in 20 ml. of absolute ethanol and stored at 3°. After 3 days, 1.4 g. of essentially racemized N-D-dulcityl-2,4-dihydroxy-3,3-dimethylbutyramide was removed by filtration, m.p. 127–137°, $[\alpha]_D$ −13°. This specific rotation corresponds to a mixture of 46% *d*- and 54% *l*-N-D-dulcityl-2,4-dihydroxy-3,3-dimethylbutyramide.

In another experiment the reaction mixture was hydrolyzed with 10% hydrochloric acid directly after removal from the reactor. The racemized pantolactone which was isolated had a specific rotation of 0°.

The Coupling of β-Alanine and D(−)-Pantolactone. The Synthesis of D(+)-Calcium Pantothenate. (a) **Calcium Metal in Methyl Cellosolve.**—In a 3-necked, round-bottomed flask equipped with a stirrer and a condenser fitted with a drying tube were placed 3.5 g. (87.3 millimoles) of calcium turnings and 200 ml. of methyl Cellosolve. The methyl Cellosolve was heated to the reflux temperature under nitrogen with stirring for 1 hr. After cooling, the flask was immersed in a constant temperature bath at 35°, and 15.6 g. (0.175 mole) of β-alanine was added and stirring was continued for 1 hr. D(−)-Pantolactone (25.2 g., 0.194 mole, 10% excess) was then added and stirring was continued at 35° for about 2 hr. Celite (1.0 g.) was added to the black solution, and it was filtered through a pressure filter in a nitrogen atmosphere to remove the black coloration. The filtrate was transferred to another 3-necked flask equipped with a mechanical stirrer and a nitrogen inlet and the flask was placed in a 35° constant temperature bath. The clear solution was seeded with D(+)-calcium pantothenate and stirred in a nitrogen atmosphere for 7 hr. The white solid which separated was removed by filtration and washed on the filter with 300 ml. of methyl Cellosolve. The product was dried in a vacuum desiccator over concentrated sulfuric acid for two days; however, this treatment did not remove the methyl Cellosolve completely. The residual solvent was removed in a vacuum oven at 50° (24 hr.). The yield of D(+)-calcium pantothenate was 36.0 g. (87%), m.p. 193.5–195°, $[\alpha]_D$ +27°. A second crop, 0.38 g. (1%), was obtained by stirring the mother liquor further at 35°, m.p. 193.5–195°.

Anal. Calcd. for $C_{18}H_{32}CaN_2O_{10}$: C, 45.36; H, 6.77; N, 5.88; Ca, 8.41. Found: C, 45.64; H, 6.85; N, 6.21; Ca, 8.23; bioassay (*Lactobacillus arabinosus*), 100%.

The filtrates were concentrated under reduced pressure to remove methyl Cellosolve. The residue was taken up in 60 ml. of 10% hydrochloric acid, and the solution was heated on a steam-bath for 4 hr. After cooling, the aqueous solution was extracted with chloroform (*ca.* 150 ml.), and the chloroform solution was dried over anhydrous sodium sulfate. The chloroform was removed under reduced pressure leaving 4.0 g. of recovered pantolactone. An aliquot of the recovered lactone, after sublimation, had a specific rotation of −48°. The yield of D(+)-calcium pantothenate based on pantolactone was 94%.

The methyl Cellosolve used in this reaction must be distilled before use to eliminate heavy metal contamination of the final product. From one lot of methyl Cellosolve which was shipped from the manufacturer in a one-gallon metal container, a 100-ml. aliquot yielded 80 mg. of residue on evaporation. After ignition, the 60 mg. of ash which was obtained contained magnesium, calcium, lead, silicon and a trace of iron. D(+)-Calcium pantothenate which was prepared in this lot of methyl Cellosolve contained 5000 p.p.m. of heavy metals whereas the U.S.P. allowable limit is 20 p.p.m.[21]

(b) **Ion Exchange Resin.**—A solution of 10 g. of β-alanine (0.113 mole) in 1000 ml. of deionized water was passed through a column of IR-410 anion exchange resin

(21) *Cf.* "U.S.P.," Vol. XV, 1955, p. 122.

(OH⁻ cycle) (¾" × 18"). The column was washed with 500 ml. of water, and the effluent was concentrated to dryness to determine the amount of β-alanine that came through the column (residue = 0 g.). The resin was washed with 1000 ml. of absolute ethanol followed by dry ether. A vacuum was then applied to the column to dry the resin. The dried resin was transferred to a 1-l. erlenmeyer flask, and 32.5 g. (0.25 mole) of D(−)-pantolactone in 200 ml. of absolute ethanol was added. The flask was gently shaken for 67 hr. at 25°. The resin was removed by filtration and washed well with absolute ethanol followed by dry ether. The filtrate was concentrated to dryness yielding 9.0 g. (50% recovery of the theoretical excess) of pantolactone. Calcium chloride (6.2 g., 0.56 mole) in 200 ml. of water was passed through the resin (in the form of a column again) followed by 500 ml. of deionized water. The effluent was concentrated to dryness yielding a sirup-like residue, 31.7 g., which was dried by distillation of absolute ethanol. It was dissolved in hot methanol, seeded with a trace of D(+)-calcium pantothenate and set aside at room temperature overnight. The white solid that formed on standing was removed by filtration, 13.0 g. (49% yield), m.p. 226–230° dec., $[\alpha]_D$ +21°. A bioassay with *L. arabinosus* indicated that this material was 67% D(+)-calcium pantothenate.

Anal. Calcd. for calcium pantothenate, $C_{18}H_{32}CaN_2O_{10}$: C, 45.36; H, 6.77; N, 5.88; Ca, 8.41. Found: C, 44.56; H, 6.85; N, 3.97; Ca, 9.57.

From the bioassay and the analytical data, it was obvious that the product was not D(+)-calcium pantothenate but was instead a mixed salt, calcium pantothenate-pantoate. Calcd. for $C_{15}H_{27}CaNO_9$: C, 44.43; H, 6.71; N, 3.46; Ca, 9.89. Found: C, 44.56; H, 6.85; N, 3.97; Ca, 9.57. The calcium pantothenate portion of the molecule represents about 64% of its total weight, in good agreement with the 67% activity determined in the bioassay.

A second crop of solids (4.1 g., 15.4%) was obtained after storage for an additional 24 hr. which proved to be D(+)-calcium pantothenate, m.p. 193–196°. Bioassay with *l. arabinosus* indicated a purity of 90%.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Pyridine-1-oxides. III. Oxidative Coupling of 4-Nitro-3-picoline-1-oxide[1]

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4-Nitro-3-picoline-1-oxide (I) undergoes a facile oxidative coupling upon treatment in alkaline medium with oxygen, sodium nitrite or *n*-butyl nitrite to give 4,4′-dinitro-1,1′-dioxy-3,3′-dipicolyl (II). Several reactions of II are discussed.

In the course of an investigation of the chemistry of pyridine-1-oxides, 4-nitro-3-picoline-1-oxide (I) was prepared and utilized as a key intermediate for the preparation of derivatives of nicotinic acid[3] and for a new synthesis of the alkaloid ricinine.[4] In the hope of extending further the usefulness of this versatile intermediate, an attempt was made to prepare 4-nitro-3-pyridyl aldehyde *via* the corresponding oxime by treatment of 4-nitro-3-picoline-1-oxide with *n*-butyl nitrite in the presence of a molar quantity of sodium ethoxide in ethanol. This method is similar to that reported by Lapworth[5] for the conversion of *o*-nitrotoluene to its oxime. Upon mixing the reactants at 0°, a rapid and pronounced color change from green-blue to red-brown took place with the simultaneous separation of a yellow, alkali-insoluble product (A). The same product was also obtained, although in lower yield, when a suspension of I in water containing an excess of sodium nitrite and a molar quantity of sodium hydroxide was shaken at room temperature or when oxygen was passed through a mixture of I in aqueous sodium hydroxide or in sodium ethoxide in ethanol.

The anticipated oxime structure for the product A was immediately suspect on the basis of its insolubility in alkali. Microanalytical values were almost identical with those calculated for I, but a molecular weight determination revealed that the product A was actually a "dimer" of I. An oxidative coupling had thus taken place to give 4,4′-dinitro-1,1′-dioxy-3,3′-dipicolyl (II) (A) under conditions reminiscent of the oxidative coupling of *o*- and *p*-nitrotoluenes to the corresponding bibenzyls.[6–14] It is noteworthy that treatment of 4-nitropyridine-1-oxide with sodium nitrite and sodium hydroxide leads only to reduction to the corresponding azo compound,[15] and treatment of 4-nitro-2-picoline-1-oxide with sodium alkoxides results in normal nucleophilic displacement of the nitro group.[16]

It is curious that the formation of II from 4-nitro-3-picoline-1-oxide (I) has not been noted previously. Thus, Katritzky[17] treated I with sodium benzylate in benzyl alcohol and noted the formation of an intense green coloration (similar to that

(1) This work was supported in part by grants from the Smith, Kline and French Foundation and from the National Science Foundation.

(2) Parke, Davis and Co. Fellow, 1954–1955.

(3) E. C. Taylor and A. J. Crovetti, *J. Org. Chem.*, **19**, 1633 (1954).

(4) E. C. Taylor and A. J. Crovetti, THIS JOURNAL, **78**, 214 (1956).

(5) A. Lapworth, *J. Chem. Soc.*, 1265 (1901).

(6) O. Fischer and E. Hepp, *Ber.*, **26**, 2231 (1893).

(7) A. Reissert, *ibid.*, **30**, 1030 (1897).

(8) A. G. Green, A. H. Davies and R. S. Horsfall, *J. Chem. Soc.*, **91**, 2076 (1907).

(9) A. K. Plisov, *Ukrainskii Khem. Zhur.*, **4**, Sci. Pt., 241 (1929); *C. A.*, **24**, 1108 (1930).

(10) R. C. Fuson and H. O. House, THIS JOURNAL, **75**, 1325 (1953).

(11) G. R. Yohe, D. R. Hill, J. E. Dunbar and F. M. Scheidt, *ibid.*, **75**, 2688 (1953).

(12) R. Oda and T. Tsuruta, *Repts. Chem. Research Inst.*, Kyoto Univ., **16**, 6 (1947); *C. A.*, **46**, 950 (1952).

(13) T. Tsuruta, R. Nagatomi and J. Furukawa, *ibid.*, **30**, 47 (1952); *C. A.*, **48**, 13376 (1954).

(14) T. Tsuruta, T. Fueno and J. Furukawa, THIS JOURNAL, **77**, 3265 (1955).

(15) E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).

(16) I. Suzuki, *J. Pharm. Soc. Japan*, **68**, 126 (1948); *C. A.*, **47**, 8074 (1953).

(17) A. R. Katritzky, *J. Chem. Soc.*, 2404 (1956).