[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Studies on Antipodes. VIII.[1] Synthesis of a Series of Valine Derivatives[2]

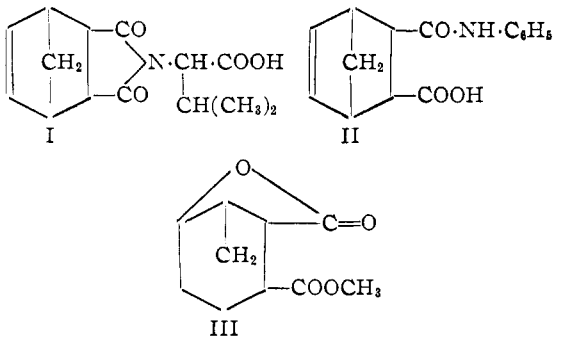
BY SIDNEY W. FOX AND FREDERICK N. MINARD[3]

A number of new derivatives of valine have been synthesized and characterized. Many of the acyl substituents contained an ethylenic linkage alpha to a carbonyl group; other acyl groups were of the norcamphane type. None of the compounds showed either pronounced antibacterial activity, or antipodal specificity in those cases in which the optical forms were prepared and compared.

The valine derivatives reported in this paper were prepared as part of a study of structural modifications of D-amino acids and of the effects of the products on inhibition of,[1] and utilization by,[4] bacteria.

A number of the derivatives prepared contained within their structures an ethylenic linkage alpha to a carboxyl group. This selection was made on the basis of the known enzyme-inactivating properties of compounds of this type.[5] The ethenoid substituents included maleyl, itaconyl and fumaryl groups.

A number of valine derivatives in the norcamphane series has been synthesized for two reasons. One of these considerations was the objective of preparing compounds containing multiple rings as in penicillin; the other was the claim for insecticidal activity in the corresponding condensation production of *endo-cis*-3,6-*endo*-methylene-Δ^4-tetrahydrophthalic anhydride with amylamine.[6] Structures which represent some of the compounds in this group, as described in the text, are



None of the compounds prepared in this work showed pronounced activity or antipodal specificity in preliminary tests on inhibition of bacterial growth. A considerable list of inhibitions of microbial growth by unsubstituted D-amino acids has now been recorded.[7-16,1] Such inhibitions are not readily recognized under usual cultural conditions and need to be systematically analyzed in each case. These facts may explain a conclusion, on the rarity of such inhibitions,[17] which appeared before most of the cited results were registered in the literature.

Similarly, one may not expect frequent discovery of antibacterial D-amino acid derivatives. Antibiotic structures are undoubtedly highly specific and the D-residues, by definition, contribute to the specific structures in those antibiotics in which they are found. The exact nature of such contribution and essential accompaniments is yet to be clarified.

Information on the bacterial utilizability of some of these derivatives is being organized for presentation at a later date.

Experimental

Nitrogen analyses are by micro Kjeldahl. Carbon and hydrogen analyses were performed by Clark Microanalytical Laboratory, Urbana, Illinois.

Maleyl-DL-valine, Maleyl-D-valine and Maleyl-L-valine.—The following procedure was adapted from that reported for the preparation of maleylglycine.[18] A mixture of 10.9 g. (0.110 mole) of maleic anhydride and 11.7 g. (0.100 mole) of DL-valine was heated at 100° for three-fourths of an hour during which time the powdery mass was often agitated. It was then cooled, triturated in dilute hydrochloric acid, and filtered. This material was dissolved in 50 ml. of hot ethanol, 100 ml. of hot water was added, and the solution was allowed to crystallize in the cold. Filtration gave a yield of 15.5 g. (69%) of maleyl-DL-valine with a m.p. of 165–167°. Two other recrystallizations from the same solvents produced a material with a m.p. of 166–167°.

Anal. Calcd. for $C_9H_{13}O_5N$: neut. equiv., 108; N, 6.51. Found: neut. equiv., 108, 109; N, 6.51.

When the fusion of maleic anhydride and either optical isomer of valine was carried out at 100° as for the above racemic compound, an intractable oil resulted. A mixture of 5.8 g. (0.050 mole) of either D- or L-valine and 5.9 g. (0.060 mole) of maleic anhydride was kept at 70° and agitated until the mush changed to a solid. This required from 15 to 20 minutes. The solid was dissolved in 200 ml. of hot ethyl acetate and then carbon tetrachloride was added to incipient precipitation. A seed was introduced and the mixture was set aside to crystallize in the cold. The yields were about 75% of theory. The melting points were 132–133° after several other recrystallizations from the same solvents.

The $[\alpha]^{25}D$ of the maleyl-D-valine in alcohol (3%) was only $+1.6 \pm 0.1°$. In ethyl acetate (3%), however, $[\alpha]^{21}D$ $-26.2 \pm 0.3°$.

Anal. Calcd. for $C_9H_{13}O_5N$: neut. equiv., 108; N, 6.51. Found: neut. equiv., 108; N, 6.45.

(1) Paper VII, S. W. Fox and Y. Kobayashi, THIS JOURNAL, **73**, 353 (1951).

(2) Journal Paper No. J-1907 of the Iowa Agricultural Experiment Station, Project 980.

(3) Upjohn Co. Fellow, 1946–1949. This work is from the Ph.D. thesis of Frederick N. Minard, Iowa State College, 1949.

(4) S. W. Fox, Y. Kobayashi, S. Melvin and F. N. Minard, THIS JOURNAL, **70**, 2404 (1948).

(5) W. B. Geiger and J. E. Conn, *ibid.*, **67**, 112 (1945).

(6) E. W. Bousquet, U. S. Patent 2,424,220 (July 22, 1947).

(7) S. W. Fox, M. Fling and G. N. Bollenback, *J. Biol. Chem.*, **155**, 465 (1944).

(8) M. Fling and S. W. Fox, *ibid.*, **160**, 329 (1945).

(9) G. W. Kidder and V. C. Dewey, *Proc. Natl. Acad. Sci.*, **33**, 347 (1947).

(10) Y. Kobayashi, M. Fling and S. W. Fox, *J. Biol. Chem.*, **174**, 391 (1948).

(11) E. Jeney, *Hung. Acta Physiol.*, **1**, 142 (1948).

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(13) J. Nicolle, *Compt. rend.*, **229**, 252 (1949).

(14) B. D. Davis and W. K. Maas, THIS JOURNAL, **71**, 1865 (1949).

(15) J. Nicolle, *Compt. rend.*, **231**, 1002 (1950).

(16) R. G. Chitre, R. J. Sirny and C. A. Elvehjem, *Arch. Biochem. Biophys.*, **31**, 398 (1951).

(17) G. M. Hills, *Biochem. J.*, **45**, 369 (1949).

(18) H. Wurbin and P. E. Spoerri, THIS JOURNAL, **69**, 1682 (1947).

For maleyl-L-valine, $[\alpha]^{21}D$ +25.5 ± 0.5° in ethyl acetate (3%).

Anal. Calcd. for $C_9H_{13}O_5N$: neut. equiv., 108; N, 6.51. Found: neut. equiv., 109; N, 6.50.

Fumaryl-di-DL-valine.—Eleven and seven-tenths grams (0.10 mole) of DL-valine was dissolved in 100 ml. (0.10 mole) of 1.0 *N* sodium hydroxide. To this solution there were added, with vigorous shaking and in several alternate portions, 7.7 g. (0.050 mole) of fumaryl chloride (Eastman Kodak Co.) dissolved in 50 ml. of ether and 100 ml. (0.10) of 1.0 *N* sodium hydroxide. Each solution had been cooled in ice. After the odor of fumaryl chloride had disappeared, the aqueous layer was separated and acidified with concentrated hydrochloric acid. The resulting precipitate was filtered off and dried. It was recrystallized by solution in 200 ml. of warm water. After cooling overnight in the refrigerator there was obtained 10 g. (64%) of fumaryl-di-DL-valine with a m.p. (copper block) of 266–267°. The m.p. rose to 282–283° after several other recrystallizations from the same solvent.

Anal. Calcd. for $C_{14}H_{22}O_6N_2$: neut. equiv., 157; N, 8.91. Found: neut. equiv., 158; N, 8.70.

Ethyl Itaconoyl-DL-valinate.—A solution of 23.5 g. (0.210 mole) of itaconic anhydride[19] in anhydrous ether was added to a dried ether solution of the free base prepared from 44.0 g. (0.242 mole) of DL-valine ethyl ester hydrochloride. The total volume was about 500 ml. After the solution had stood for three days, the ether was evaporated and the residue was triturated in cold water until it solidified. Filtration gave 37.5 g. (70%) of a product with a m.p. of 79–83°. Repeated recrystallization from hot water brought the m.p. to 88–89°. Whether the methylene group is in the α or β position relative to the amide group was not determined.

Anal. Calcd. for $C_{12}H_{19}O_5N$: neut. equiv., 257; N, 5.44. Found: neut. equiv., 256; N, 5.44.

Itaconoyl-DL-valine.—Ethyl itaconoyl-DL-valinate (1.00 g., 0.0039 mole) was dissolved in 8.0 ml. (0.01 mole) of 1.85 *N* sodium hydroxide solution and the liquid was allowed to stand for five hours. It was then acidified and evaporated to dryness under reduced pressure at room temperature. The residue was extracted several times with hot absolute ethanol. Evaporation of the alcohol left a residue which soon crystallized; m.p. 123–128°. This material was best purified by several recrystallizations from water, until the m.p. attained the value of 139–140°.

Anal. Calcd. for $C_{10}H_{15}O_5N$: neut. equiv., 115; N, 6.11. Found: neut. equiv., 115; N, 6.04.

***endo-cis*-3,6-Endomethylene-Δ^4-tetrahydrophthaloyl-DL-valine (I).**—Eight and two-tenths grams (0.050 mole) of *endo-cis*-3,6-endomethylene-Δ^4-tetrahydrophthalic anhydride (Eastman Kodak Co. bicyclo[2,2,1]5-heptene-2,3-dicarboxylic anhydride) and 5.8 g. (0.050 mole) of DL-valine were fused at 150° until all the water distilled (20 min.). Several recrystallizations from carbon tetrachloride–hexane gave crystals with m.p. 118–119°.

Anal. Calcd. for $C_{14}H_{17}O_4N$: neut. equiv., 263: N, 5.32. Found: neut. equiv., 264, 265; N, 5.39.

***endo-cis*-3,6-Endomethylene-Δ^4-tetrahydrophthaloyl-D-valine.**—Eight and two-tenths grams (0.050 mole) of *endo-cis*-3,6-endomethylene-Δ^4-tetrahydrophthalic anhydride was fused with 5.8 g. (0.050 mole) of D-valine as described above for the racemic isomer. After repeated recrystallization from benzene–cyclohexane there were obtained crystals of m.p. 116–117°; $[\alpha]^{24}D$ +60.4° ± 0.5° (3% in ethanol).

Anal. Calcd. for $C_{14}H_{17}O_4N$: neut. equiv., 263; N, 5.32. Found: neut. equiv., 264; N, 5.35.

***endo-cis*-3,6-Endomethylene-Δ^4-tetrahydrophthalanilic acid (II).**—Earlier attempts to prepare this compound were reported to be unsuccessful,[20] and to produce only the imide. The failure was probably due to the use of hot acetic acid for purification of the reaction product between aniline and the bicyclic anhydride.

To a solution of 4.1 g. (0.025 mole) of *endo-cis*-3,6-*endo*-methylene-Δ^4-tetrahydrophthalic anhydride in 20 ml. of benzene there was added, at room temperature, a solution of 2.5 ml. (0.030 mole) of aniline in 10 ml. of benzene. Crystals of the anilide soon appeared and after cooling they were filtered off to give a good yield of the desired acid. These crystals were purified at room temperature from ethyl acetate–hexane to a m.p. of 135–145°, depending upon the rate of heating.

The anilide rapidly formed the corresponding imide in aqueous solution. This was indicated by the fact that following neutralization by sodium hydroxide to a phenolphthalein end-point the solution soon became more basic, even when kept in ice. For this reason no attempt was made to hydrolyze either I or II to the diacid.

Anal. Calcd. for $C_{15}H_{15}O_3N$: neut. equiv., 257; N, 5.44. Found: neut. equiv., 261; N, 5.43.

2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzanilide γ-Lactone.—Two experiments were carried out to determine the reactivity of the lactone ring of 4-hydroxy-*cis*-3,6-endomethylenehexahydrophthalic acid γ-lactone[21] toward amines.

A mixture of 0.50 g. (0.0027 mole) of the lactonic acid and 0.70 g. (0.0034 mole) of phosphorus pentachloride was agitated in 10 ml. of benzene until all the solids were in solution. Dry air was passed through this solution to remove the hydrogen chloride gas, and then a cold solution of 2.0 ml. (0.021 mole) of aniline in 10 ml. of benzene was added. After the mixture had stood at room temperature for 15 minutes, the solid material was filtered off and slurried successively with dilute hydrochloric acid, dilute sodium carbonate solution, and finally with water. The anilide, having a m.p. of 232–233°, was obtained after several recrystallizations of the residue from butanol.

Anal. Calcd. for $C_{15}H_{15}O_3N$: N, 5.44. Found: N, 5.46.

The anilide was also obtained by the fusion of the lactonic acid with aniline at 200° for two hours. This indicated that, due to the fixed ring system, the lactone ring is extremely unreactive toward amines.

DL-Valine Methyl Ester Hydrochloride.—A mixture of 30 g. of DL-valine and 300 ml. of methanol was saturated with dry hydrogen chloride, refluxed for two hours, resaturated with hydrogen chloride, and refluxed 15 minutes longer. Evaporation under reduced pressure over steam gave the methyl ester hydrochloride as an oil which crystallized only after the complete removal of the excess hydrogen chloride. The resulting solid was pure enough for further reactions.

For analytical purposes the material was purified by solution in acetone, addition of chloroform, and precipitation by hexane. The m.p. remained in the range 90–97°.

Anal. Calcd. for $C_6H_{14}O_2NCl$: N, 8.36; Cl, 21.2. Found: N, 8.44; Cl (gravimetric), 21.4, 21.4.

DL-Valine *n*-Butyl Ester Sulfate.—The *n*-butyl ester of DL-valine, although not used, was prepared by a Fischer esterification according to the procedure of Morgan.[22] This ester was carefully added to a solution of concentrated sulfuric acid in anhydrous ether until the oil, which first formed, had solidified. The solid was recrystallized from acetone–ether to a m.p. 140.5–141.5°.

Anal. Calcd. for $C_{18}H_{38}O_4N_2 \cdot H_2SO_4$: N, 6.30; SO_4, 21.6. Found: N, 6.15; SO_4 (gravimetric), 21.6, 21.7.

Methyl Ester of 2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzoyl-DL-valine γ-lactone.—The acid chloride of the lactonic acid was prepared in 15 ml. of benzene from 3.6 g. (0.020 mole) of the acid and 4.6 g. (0.022 mole) of phosphorus pentachloride. The benzene and phosphorus oxychloride were removed from the solution under reduced pressure in a water-bath at 60°. To the cooled residue there was added a cold benzene solution of the base prepared from 8.3 g. (0.050 mole) of DL-valine methyl ester hydrochloride. The mixture was allowed to stand at room temperature for several hours, and it was then washed once with water, dilute hydrochloric acid, and finally with dilute sodium carbonate solution. The benzene solution was dried over sodium sulfate and then evaporated under reduced pressure to leave a residue of 4.3 g. (73%) of the methyl ester. Repeated purification from chloroform–hexane gave a product with a m.p. of 168–169°.

Anal. Calcd. for $C_{15}H_{21}O_5N$: C, 61.0; H, 7.17; N, 4.74. Found: C, 61.3, 61.1; H, 6.96, 6.81; N, 4.71.

(19) R. L. Shriner, S. G. Ford and L. J. Roll, in A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 368.

(20) M. S. Morgan, R. S. Tipson, A. Lowy and W. E. Baldwin, THIS JOURNAL, **66**, 404 (1944).

(21) K. Alder and G. Stein, *Ann.*, **514**, 22 (1934).

(22) W. T. J. Morgan, *J. Chem. Soc.*, 79 (1926).

2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzoyl-DL-valine γ-Lactone.—Two and eight-tenths grams (0.0095 mole) of the above methyl ester was added to 9.8 ml. (0.020 mole) of 1.95 *N* sodium hydroxide, and the mixture was warmed on the steam-bath for a few minutes until complete solution. The liquid was cooled and then acidified with concentrated hydrochloric acid. The mixture was then filtered to give 2.4 g. (89%) of material with m.p. of 187–189°. The m.p. rose to 191.5–192.5° after three recrystallizations from water.

Anal. Calcd. for $C_{14}H_{19}O_5N$: C, 59.8; H, 6.81; N, 4.98; neut. equiv., 281. Found: C, 59.7; H, 6.49; N, 4.94; neut. equiv., 280.

Methyl Ester of 2-Carboxy-4-hydroxy-*cis*-3,6-endomethylenehexahydrobenzoic Acid γ-Lactone (III).—Alder and Stein[23] isolated this ester in a 15% yield following esterification with sulfuric acid as a catalyst. The use of hydrogen chloride proved to be more satisfactory.

A mixture of 20 g. (0.11 mole) of the previously described *cis*-lactonic acid and 200 ml. of methanol was saturated with dry hydrogen chloride and refluxed for one hour. It was then resaturated with hydrogen chloride and refluxed for a total of three hours. The residue from evaporation of the methanol was dissolved in chloroform and extracted once with water and once with potassium carbonate solution. The chloroform solution of the ester was dried over sodium sulfate and evaporated to give a residue of 18.5 g. (86%) of the methyl ester with a m.p. of 80–82°. The m.p. was raised to 82–83° after several recrystallizations from ethyl acetate–hexane. The previously reported value[17] was 85°.

(23) K. Alder and G. Stein, *Ann.*, **514**, 24 (1934).

2-Carboxy-4-hydroxy-*trans*-3,6-endomethylenehexahydrobenzoyl-DL-valine γ-Lactone.—The acid chloride of the lactonic acid[21] was prepared in benzene from 5.0 g. (0.027 mole) of the acid prepared in turn by saponification of the above methyl ester and 6.0 g. (0.029 mole) of phosphorus pentachloride. The benzene and the phosphorus oxychloride were removed from the solution under reduced pressure in a water-bath kept at 60°. Small amounts of benzene were then added and evaporated to ensure removal of all phosphorus oxychloride. To the residue there was added, with vigorous shaking, a cold solution of 6.5 g. (0.055 mole) of DL-valine in 50 ml. (0.10 mole) of 2 *N* potassium carbonate. The resulting solution was acidified in the cold with concentrated hydrochloric acid. Upon the addition of about 20 ml. of ethyl acetate to the oily mixture, there appeared a precipitate which was filtered off and dried; m.p. 210–218°. The material was purified at room temperature by solution in absolute ethanol, addition of ethyl acetate to prevent layering, and then precipitation with hexane. Repetition of this procedure finally gave crystals with a m.p. 230–231°.

Anal. Calcd. for $C_{14}H_{19}O_5N$: C, 59.8; H, 6.81; N, 4.98; neut. equiv., 281. Found: C, 59.9; H, 6.42; N, 4.88; neut. equiv., 281.

Antibacterial Tests.—The compounds were tested against *Escherichia coli* in the medium of Gray and Tatum[24] and against *Lactobacillus arabinosus* in a "synthetic" medium[7] at concentrations of substance of 1.00, 0.100, and 0.010 mg./ml., by Daniel Atkinson. All tubes showed at 20 hr. virtually the same growth as in controls.

(24) C. H. Gray and E. L. Tatum, *Proc. Natl. Acad. Sci.*, **30**, 404 (1944).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA]

The Degradation of Sugars by Means of their Disulfones[1]

BY D. L. MACDONALD AND HERMANN O. L. FISCHER

When D-glucose diethyl mercaptal pentaacetate was oxidized with monoperphthalic acid in ether, D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I) was the main product of the reaction. When this was treated wtih hydrazine in methanol, a splitting of the molecule took place, and D-arabinose and bis-(ethanesulfonyl)-methane could be isolated in good yield. In an analogous manner, D-lyxose could be prepared from D-galactose. The double bond in the *arabo*-stereoisomer readily adds ammonia, to give predominantly a disulfone derivative of the glucosamine series.

In his first paper on sugar mercaptals, Emil Fischer[2] described briefly the attempted oxidation of these compounds to the disulfones using potassium permanganate. Since that time nothing appears to have been published on this type of sugar derivative, although the thioglycosides have been oxidized to the corresponding sulfoxides[3] and some monosulfones of sugars have been prepared and their properties studied.[4]

If the acetylated mercaptals of glucose, mannose or galactose are oxidized with monoperphthalic acid in ether, readily crystallizing 1,1-disulfones can be obtained in yields varying with the particular sugar employed. The present work deals with the disulfones derived from the above mentioned hexoses, and at the time that the results of this work were first announced,[1] it was learned that Seidman and Link[5] had oxidized sugar mercaptals with peracetic acid because of their desire to investigate the pharmacological properties of sulfone-containing sugar derivatives.

When D-glucose diethyl mercaptal pentaacetate was oxidized in our laboratory with monoperphthalic acid in ether, the main product was a compound, isolated in a yield of 60–70%, whose analyses corresponded with the formula of an unsaturated disulfone, D-*arabo*-3,4,5,6-tetraacetoxy-1,1-bis-(ethanesulfonyl)-hexene-1 (I). Tests for the presence of a double bond proved negative with tetranitromethane and, as expected, with bromine in acetic acid; however, the compound slowly gave a positive test with osmium tetroxide. The presence of a double bond was further substantiated by the isolation of the same compound in a yield of 55–60% by oxidation of D-mannose diethyl mercaptal pentaacetate. The analogous oxidation of D-galactose diethyl mercaptal pentaacetate likewise gave in good yield (85%) an unsaturated disulfone,

(1) Presented in part before the Division of Sugar Chemistry at the 118th National Meeting of the American Chemical Society, Chicago, Illinois, September, 1950.

(2) Emil Fischer, *Ber.*, **27**, 673 (1894).

(3) F. Micheel and H. Schmitz, *ibid.*, **72**, 992 (1939).

(4) F. Wrede and W. Zimmerman, *Z. physiol. Chem.*, **148**, 65 (1925); W. A. Bonner and R. W. Drisko, THIS JOURNAL, **70**, 2435 (1948); **73**, 3699, 3701 (1951).

(5) M. Seidman, Ph.D. Thesis, Summaries of Doctoral Dissertations, University of Wisconsin Press, Madison, Wisconsin, Vol. 12, 1950–1951.