2-oxaloglutarate, 50 ml. of 95% ethanol, and 18 mg. of platinum dioxide were shaken in hydrogen under a pressure of 45 lbs./sq. in. at room temperature. The solution was filtered, evaporated under reduced pressure, and the light yellow residual oil was dissolved in 50 ml. of ether. The solution was washed 3 times with 5 ml. of 10% potassium carbonate solution to remove any unchanged compound, dried over anhydrous sodium sulfate, and evaporated under reduced pressure, yielding 2.8 g. of a residual oil having a fruit-like odor; on distillation, there was obtained a colorless liquid boiling at 140–141° (0.005 mm.) and a reddish oily residue.

Anal. Calcd. for $C_{13}H_{22}O_7$ (XI): Sapon. equiv., 96.8. Found: Sapon. equiv., 96.54.

Two and four-tenths g. of the undistilled triethyl 2-hydroxy-3-carboxyadipate was refluxed 2 hr. with 26.2 ml. (about an equivalent quantity) of 0.9*N* NaOH. The solution was acidified with a slight excess of *N* HCl, extracted continuously with ether for about 100 hr., and the ether solution was evaporated to dryness under reduced pressure. The residue was dissolved in water, and evaporated again to dryness to remove traces of hydrochloric acid. The 1.5 g. of residual sirup, after 3 days over phosphorus pentoxide in a vacuum desiccator, solidified. On repeated recrystallization from acetone and benzene, white crystals melting at 127–129° were obtained.

Anal. Calcd. for $C_7H_{10}O_7$: C, 40.78; H, 4.89; neut. equiv., 68.7. Found: C, 40.50; H, 4.88; neut. equiv., 68.3.

Reduction of triethyl 2-oxaloglutarate with sodium borohydride. To a solution of 5.1 g. of sodium borohydride in 25 ml. of water, a solution of 12 g. of triethyl 2-oxaloglutarate in 50 ml. of 70% methanol was added dropwise while stirring and cooling with ice water, and stirring was continued for 20 min. at room temperature. The reaction mixture was added to 2*N* H_2SO_4 to bring the *p*H to 3, filtered, evaporated at room temperature under vacuum, and extracted several times with ether after saturation with sodium chloride; then the ether solution was washed with 10% potassium carbonate solution until the latter gave a negative test for the carbonyl group with 2,4-dinitrophenylhydrazine, and dried over anhydrous sodium sulfate. Evaporation of the ether yielded 4.3 g. of oil. This was refluxed for 2 hr. with 17 ml. of *N* NaOH, neutralized with 17 ml. of *N* HCl with cooling, and the solution was evaporated to dryness under vacuum. The residue was extracted with acetone, the acetone solution was concentrated, and benzene was added until turbidity occurred. On standing overnight in the refrigerator crystals were precipitated which, after being recrystallized twice from acetone and benzene, melted at 127–129° and did not depress the melting point of the product obtained by catalytic reduction.

Trianilide of 2-hydroxy-3-carboxyadipic acid. Two-tenths g. of 2-hydroxy-3-carboxyadipic acid was neutralized with *N* NaOH, and the solution was evaporated to dryness and powdered. The dry sodium salt was heated 1 hr. at 150–160° with 1 ml. of aniline and 0.3 ml. of concentrated hydrochloric acid. After addition of 10 ml. of 2*N* HCl, the mixture was filtered, washed with water, and recrystallized 3 times from acetic acid; white crystals melting at 251° with decomposition were obtained in a yield of 0.15 g.

Anal. Calcd. for $C_{25}H_{25}N_3O_4$: C, 69.59; H, 5.84; N, 9.74. Found: C, 69.20; H, 5.92; N, 9.60.

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PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

Synthesis of the Racemic and Optically Active Forms of α-Amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric Acid[1]

HOWARD E. SMITH[2] AND J. MURRAY LUCK

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In the continuing search for chemotherapeutic agents for the treatment of cancer, the DL-, D-, and L-α-amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric acids were synthesized. Resolution of the intermediate α-acetamido-γ-*p*-nitrophenylbutyric acid as the (+)- and (−)-α-phenylethylamine salts led to the optically active isomers, the absolute configurations of which were tentatively inferred from their different degrees of biological activity; *i.e.* the L isomer caused a prompt transitory regression of a Cloudman malignant melanoma, S 91, in male mice while the D isomer caused only a barely perceptible brief regression.

Introduction. The carcinostatic and carcinolytic properties of nitrogen mustards, di(β-chloroethyl)-amino compounds, have been recognized for many years,[3] and recently the *p*-di(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines (I) have been prepared[4,5] and have displayed promising results in the treatment of certain types of tumors.[6] Interestingly, the L isomer, the absolute configuration of which was known by synthesis from L-phenylalanine,[4] showed a much greater ability to inhibit the growth of these tumors than did the D isomer.[6] This was one of the first examples of selectivity through optical isomerism with agents of this kind.

(1) A short summary of these syntheses has appeared previously (J. M. Luck, *Cancer Research*, **17**, 1071 (1957), and the compounds have also been named 2-amino-4-*p*-di(2-chloroethyl)aminophenylbutyric acids.

(2) To whom inquiries concerning this communication should be sent. Present address: Organisch-Chemisches Laboratorium der E. T. H., Zurich, Switzerland.

(3) A rather complete survey of the nitrogen mustard literature may be found in J. W. Beattie and L. H. Howells, *Quart. J. Med.*, **23**, 231 (1954).

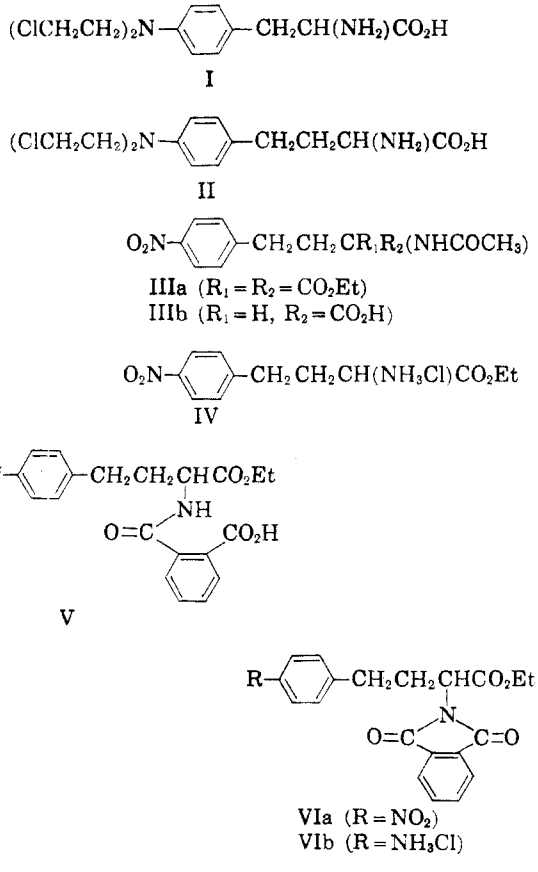
(4) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954).

(5) F. Bergel, V. C. E. Burnop, and J. A. Stock, *J. Chem. Soc.*, 1223 (1955).

(6) F. Bergel and J. A. Stock, *Ann. Rep. Brit. Emp. Cancer Camp.*, **31**, 6 (1953).

For further study of this selectivity of optical-isomers, it was desirable to determine the nature of the effect that would be produced in the carcinol static and carcinolytic properties of the *p*-di(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines by the interposition of an additional methylene group between the site of optical activity and the *p*-di(β-chloroethyl)aminophenyl moiety. With this in view the DL-, D-, and L-α-amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric acids (II) were synthesized and their carcinostatic and carcinolytic properties studied and compared with those of the *p*-di(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines. The syntheses of the butyric acid derivatives are herein described while the details concerning their biological activities are presented elsewhere.[1]

In Table I are presented the yields, physical properties, elemental analyses, and crystallization solvents for the DL-, D-, and L-α-amino-γ-*p*-di(β-chloroethyl)aminophenylbutyric acids and the intermediates encountered in their syntheses. Except as is noted in Table I, included with each compound is a number referring to a reference in the text wherein may be found the standard procedure used to obtain the compound in question. Any deviations from these procedures are fully discussed in the section below. Not included in Table I are the descriptions of the (+)- and (−)-α-phenylethylamine salts of the D- and L-α-acetamido-γ-*p*-nitrophenylbutyric acids (IIIb) since these are included in the experimental section with the description of the scheme for resolution of the racemic acid.



DISCUSSION

The synthesis of the DL-, D-, and L-α-amino-γ-*p*-di-(β-chloroethyl)aminophenylbutyric acids(II) was accomplished by a route similar to that employed by Bergel, Burnop, and Stock[5] in the synthesis of the *p*-di-(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines (I). In this present work β-*p*-nitrophenylethyl bromide[7] was condensed[8] with diethyl acetamidomalonate[9] in the presence of sodium ethoxide to yield diethyl acetamido-(β-*p*-nitrophenylethyl)malonate (IIIa). Hydrolysis of the latter compound in aqueous sodium carbonate followed by decarboxylation on acidification with hydrochloric acid[5,10] gave the racemic α-acetamido-γ-*p*-nitrophenylbutyric acid (IIIb) which was resolved into its two enantiomorphs by fractional crystallization from ethanol of the (+)- and (−)-α-phenylethylamine salts. Of the five bases with which resolution was attempted only the two α-phenylethylamines gave crystalline salts while the salts of brucine, strychnine, and cinchonine were obtained as uncrystallizable oils.

It must be mentioned that the hydrolysis of diethyl acetamido-(β-*p*-nitrophenylethyl)malonate with aqueous sodium bicarbonate was much more rapid and was accompanied by greater decomposition than is usually reported[5,10] for this reaction. The reaction was found to be complete in six hours and after decarboxylation with acid the product could be obtained in a yield of seventy-eight percent. There was some decomposition during the hydrolysis and the crystalline product invariably had an orange color which was not easily removed by recrystallization from water (charcoal). Recrystallization of this colored material from ethyl acetate or chloroform-ethanol gave the compound as light yellow plates, the melting point of which was unchanged. If the heating period for hydrolysis was extended beyond six hours, extensive decomposition occurred and an inert atmosphere (nitrogen) over the reaction did not reduce the amount of decomposition. When the heating time was extended to twenty-four hours, the time required[5] for hydrolysis of diethyl α-acetamido-*p*-nitrobenzylmalonate, a very impure product in low yield was obtained.

After decomposition with aqueous sodium hydroxide of the α-phenylethylamine salts of the D- and L-α-acetamido-γ-*p*-nitrophenylbutyric acid, the D and L acids as well as the racemic modification

(7) E. L. Foreman and S. M. McElvain, *J. Am. Chem. Soc.*, **62**, 1435 (1940).

(8) D. F. Elliott and C. R. Harington, *J. Chem. Soc.*, 1374 (1949).

(9) H. R. Snyder and C. W. Smith, *J. Am. Chem. Soc.*, **66**, 350 (1944).

(10) N. F. Alberton, *J. Am. Chem. Soc.*, **72**, 1396 (1950).

TABLE I

YIELDS, PHYSICAL PROPERTIES, AND ELEMENTAL ANALYSES FOR DL-, D-, AND L-α-AMINO-γ-p-DI-(β-CHLOROETHYL)AMINOBUTYRIC ACIDS AND INTERMEDIATES ENCOUNTERED IN THEIR SYNTHESES

Compound No. in Text	Isomer	Reference	Yield, %[a]	M.P., °C.[b]	$[\alpha]_D$, ±2°[c]	Crystallization Solvent	Crystal Form and Color	Formula	Elemental Analysis[d] Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
IIIa		(8)	70[e]	117–119		Ethanol	Fine, colorless prism	$C_{17}H_{22}N_2O_7$	55.73	55.66	6.05	6.26
IIIb	DL	(5)(10)	78	158–160		Water	Light yellow plates	$C_{12}H_{14}N_2O_5$[f]	54.13	54.04	5.30	5.43
IIIb	D	[g]	64[h]	152–154	+36 (*c*, 1.23 in ethanol at 26°)	Water	Yellow plates	$C_{12}H_{14}N_2O_5$	54.13	54.08	5.30	5.27
IIIb	L	[g]	53[h]	152–153	−34 (*c*, 1.03 in ethanol at 26°)	Water	Large, light yellow needles	$C_{12}H_{14}N_2O_5$	54.13	53.96	5.30	5.24
IV	DL	(5)	91	174–176 (dec.)		Acetone-methanol	Fine, light yellow prisms	$C_{12}H_{17}ClN_2O_4$	49.91	50.03	5.94	6.06
IV	D	(5)	93	203–205 (dec.)	+41 (*c*, 2.00 in water at 26°)	Acetone-methanol	Fine, light yellow prisms	$C_{12}H_{17}ClN_2O_4$	49.91	49.92	5.94	6.19
IV	L	(5)	92	203–205 (dec.)	−43 (*c*, 2.03 in water at 26°)	Acetone-methanol	Fine, light yellow prisms	$C_{12}H_{17}ClN_2O_4$	49.91	49.75	5.94	6.02
V	DL	(4)	81	170		Benzene-acetone	Fine, colorless needles	$C_{20}H_{20}N_2O_7$	59.99	59.63	5.04	4.99
V	D	(4)	90	172–173	−31 (*c*, 2.43 in dioxane at 25°)	Benzene-acetone	Fine, colorless needles	$C_{20}H_{20}N_2O_7$	59.99	60.00	5.04	5.06
V	L	(4)	90	172–173	+30 (*c*, 2.66 in dioxane at 25°)	Benzene-acetone	Fine, colorless needles	$C_{20}H_{20}N_2O_7$	59.99	60.10	5.04	5.22
VIa	DL	(4)	83	99–100		n-Propanol	Light yellow prisms	$C_{20}H_{18}N_2O_6$	62.82	62.90	4.74	4.87
VIa	D	(4)	96	[i]	[j]	[i]	[i]	$C_{20}H_{18}N_2O_6$	62.82	[j]	4.74	[j]
VIa	L	(4)	90	[i]	[j]	[i]	[i]	$C_{20}H_{18}N_2O_6$	62.82	[j]	4.74	[j]
VIb	DL	(4)	78	179–183 (dec.)		Abs. ethanol	Light tan, amorphous	$C_{20}H_{21}ClN_2O_4$	61.77	61.66	5.45	5.41
VIb	D	(4)	77	185–190 (dec.)	−8 (*c*, 0.68 in 50% aq. ethanol at 18°)	[k]	Colorless, amorphous	$C_{20}H_{21}ClN_2O_4$	61.77	61.80	5.45	5.27
VIb	L	(4)	75	188–182 (dec.)	+6 (*c*, 0.67 in 50% aq. ethanol at 18°)	[k]	Light tan, amorphous	$C_{20}H_{21}ClN_2O_4$	61.77	61.86	5.45	5.33
II	DL	(4)	12	154–155 (dec.)	[l]	Methanol	Colorless, amorphous	$C_{14}H_{20}Cl_2N_2O_2$	52.67	52.37	6.32	6.04
II	D	(4)	17	156–159 (dec.)	+29 (*c*, 0.87 in 2*N* HCl at 26°)	Methanol	Colorless, amorphous	$C_{14}H_{20}Cl_2N_2O_2$	52.67	52.45	6.32	6.16
II	L	(4)	20	155–158 (dec.)	−32 (*c*, 0.86 in 2*N* HCl at 26°) −12 (*c*, 0.57 in methanol at 26°)	Methanol	Light tan, amorphous	$C_{14}H_{20}Cl_2N_2O_2$	52.67	52.44	6.32	6.10

[a] Based on the appropriate compound appearing immediately above in the table or as otherwise noted. [b] Melting points were obtained by the capillary method and are uncorrected. [c] Path lengths of one decimeter. [d] Elemental analysis by Miss M. A. DaRooge, Wayne State University, Detroit 2, Mich. [e] Based on β-*p*-nitrophenylethylbromide. [f] Neutral equivalent. Calcd.: 266. Found: 266, 264. [g] This procedure is described in the experimental section of this communication. [h] Based on DL-IIIb. [i] Obtained as an uncrystallizable oil. [j] Not determined. [k] Not purified by crystallization. [l] One sample identical in all respects with others had m.p. 166–169° (dec.).

were converted by hydrolysis in 6*N* hydrochloric acid and then esterified in 2*N* ethanolic hydrogen chloride to the DL-, D-, and L-α-amino-γ-*p*-nitrophenylbutyrate hydrochlorides (IV). The free amino group in each substance was then reblocked *via* the α-*o*-carboxybenzamido compounds (V) as the α-phthalimido group (VIa). The introduction of this blocking group was necessary since subsequent intermediates were to be treated with phosphorus oxychloride; and under the conditions of the reactions, the optically active α-acetamido compounds would be racemized whereas the α-phthalimido derivatives maintain their optical integrity.[4] The racemic ethyl α-phthalimido-γ-*p*-nitrophenylbutyrate was obtained as a crystalline solid but the optically active isomers could not be made to crystallize and were obtained as oils which after washing with sodium bicarbonate and water had infrared absorption spectra identical with that of the pure, crystalline, racemic compound. The *p*-nitro group of each isomer was then reduced in methanol-ethyl acetate to the *p*-amino function with hydrogen over platinum (platinum hydroxide on calcium carbonate), and the reduced compounds isolated as the ethyl DL-, D-, and L-α-phthalimido-γ-*p*-aminophenylbutyrate hydrochlorides (Vb). The racemic form was reprecipitated from hot ethanol as a slightly hygroscopic, amorphous, light tan solid while the optically active isomers were purified by thorough washing with dry ether and were also obtained as slightly hygroscopic, amorphous solids.

The ethyl DL-, D-, and L-α-phthalimido-γ-*p*-aminophenylbutyrate hydrochlorides were then converted by the elegant method of Bergel and Stock[4] to the respective α-amino-γ-di(β-chloroethyl)aminophenylbutyric acids(II) employing in turn diethylamine, ethylene oxide, phosphorus oxychloride, 6*N* hydrochloric acid, and aqueous sodium acetate. The pure amino acids were obtained after two reprecipitations from hot methanol as amorphous, colorless or light tan solids with appearances, physical properties, and infrared absorption spectra[11] quite similar to the *p*-di(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines (I). The crude butyric acid derivatives were heavily contaminated with sodium chloride and their purification was difficult due to their reluctant solubility in hot methanol, their reluctant reprecipitation on cooling the solvent, and the coprecipitation of sodium chloride. It must be noted that the yield in this last reaction is low (12 to 20%) not only because of the difficulties in the purification of the crude material but also because of the inherent difficulties in the hydroxyethylation and chlorination reactions. No special effort was made to improve these yields.

When the carcinostatic and carcinolytic properties of the two optically active isomers of II were studied,[1] one was found more active in its ability to induce a prompt, transitory regression of Cloudman malignant melanoma, S 91, in dba/1 male mice and was tentatively assigned, in analogy to *p*-di(β-chloroethyl)amino-L-phenylalanine, the L absolute configuration. The other isomer, to which was assigned the D absolute configuration, was about one-fifth as active as its enantiomorph at the same dosage level. With the absolute configurations of II indicated by biological activity, the absolute configurations of the intermediate compounds (IIIa through VIb) were assigned by inference, since the reactions in going from IIIa to II proceed with retention of configuration at the asymmetric center.[4,5] It should be pointed out, however, that the assignments of absolute configuration are tentative and a final decision must be based on a chemical transformation of one of the optically active compounds listed in Table I to a substance of established configuration. Applying the conclusion of Lutz and Jirgensons,[12] that for optically active α-amino acids, the specific rotation of the L isomer becomes more positive with increasing acid concentration, it is indicated that possibly the configurations of the optically active α-amino-γ-*p*-di-(β-chloroethyl)aminophenylbutyric acids are opposite to that which we have inferred from the biological tests (*cf.* II in Table I).

EXPERIMENTAL[13]

D-α-*Acetamido-γ-p-nitrophenylbutyric acid* (+)-α-*phenylethyleneamine salt.* To 18.8 g. of DL-α-acetamido-γ-*p*-nitrophenylbutyric acid (0.0707 mole) dissolved in 300 ml. of hot 95% ethanol was added 8.55 g. of (+)-α-phenylethylamine[14] (0.0707 mole) dissolved in 50 ml. of hot 95% ethanol and the mixture was allowed to cool slowly overnight at room temperature. On cooling light yellow plates were deposited and after filtration there was obtained 20.3 g. (70.5% of the total) of crystalline material with $[\alpha]_D^{27}$ +15 ± 1° (*c*, 1.17 in methanol). Two recrystallizations of this mixture from 300 ml. of 95% ethanol gave 8.5 g. of the D-α-acetamido-γ-*p*-nitrophenylbutyric acid (+)-α-phenylethylamine salt (62%) as light yellow plates with $[\alpha]_D^{23}$ +44 ± 2° (*c*, 0.96 in methanol) and m.p. 204–208° (dec.), the rotation of which was not altered by further recrystallization from ethanol. Reworking of the mother liquors pro-

(11) Infrared absorption spectra of the three isomers of II were obtained using a Perkin-Elmer Model 12c infrared spectrophotometer with sodium chloride optics and in general had bands in agreement with the assigned structure, *i.e.* (Nujol mull) 2.97 μ (w), NH; 6.2–6.3 μ (s), $COO^{\ominus}$ and aromatic ring; 6.60 μ (s) aromatic ring. The infrared spectra of the three isomers of I were obtained in the same way with material supplied by Dr. J. A. Stock and with other samples of these substances synthesized in these laboratories and also had bands in agreement with the assigned structures, *i.e.* (Nujol mull) 3.00 μ (w) NH; 6.22 μ (s), aromatic ring 6.34 μ (s), $COO^{\ominus}$; 6.65 μ (s), aromatic ring. All spectra, however, had a very weak band appearing at 5.80 to 5.86 μ which is unassigned or perhaps could be assigned to presence of a small amount of the unionized carboxylic acid group.

(12) O. Lutz and B. Jirgensons, *Ber.*, 63, 448 (1930).

(13) Melting points and elemental analyses were obtained as is indicated in Table I.

(14) A. W. Ingersoll, *Org. Syntheses*, **Coll. Vol. II,** 506 (1943).

duced an additional 0.8 g. of the salt with rotation and melting point identical with those above and this material was added to the main portion. The total yield was 68% for this diastereoisomer.

Anal. Calcd. for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50. Found: C, 62.12; H, 6.52.

L-α-Acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt. During the isolation of D-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt from 18.8 g. of DL-α-acetamido-γ-p-nitrophenylbutyric acid (0.0707 mole) and 8.55 g. (+)-α-phenylethylamine (0.0707 mole) when the mother liquors were combined and evaporated to a small volume, two types of crystals were observed to form on cooling. One type was light yellow plates which were the D-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt and very fine, long, almost colorless needles. By repeated recrystallizations from a minimum of 95% ethanol these two types of crystals were separated and there was obtained 1.4 g. of the L-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt (10%) as fine, faintly yellow needles with $[\alpha]_D^{26}$ −38 ± 2° (*c*, 1.29 in methanol) and melting point 196–200° (dec.), the rotation of which was not changed by further recrystallization from ethanol.

Anal. Calcd. for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50. Found: C, 62.22; H, 6.63.

L-α-Acetamido-γ-p-nitrophenylbutyric acid (−)-α-phenylethylamine salt. The mother liquors from the isolation of 9.3 g. of the D-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt and 1.4 g. of the L-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt from 18.8 g. of the racemic acid (0.0707 mole) and 8.55 g. of the amine (0.0707 mole) were evaporated to dryness at reduced pressure (water pump) and there was obtained 16.3 g. of solid material. To this material suspended in 100 ml. of water was added concentrated aqueous sodium hydroxide until the solid was dissolved and the α-phenylethylamine was completely separated as an oil. The amine was removed from the aqueous solution by washing with three 60-ml. portions of ether. On acidification (*p*H 1) of the aqueous solution with concentrated hydrochloric acid, the partially resolved acid separated as a crystalline solid; and after cooling overnight at 0°, 10.8 g. (0.0406 mole) was collected by filtration. To the acid dissolved in 170 ml. of hot 95% ethanol was added 4.91 g. of (−)-α-phenylethylamine[13] (0.0406 mole) dissolved in 30 ml. of hot 95% ethanol, and the solution was allowed to cool slowly overnight at room temperature. On cooling light yellow plates were deposited and after filtration and one recrystallization from 95% ethanol, there was obtained 8.2 g. of the L-α-acetamido-γ-p-nitrophenylbutyric acid (−)-α-phenylethylamine salt (67% corrected for the L-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt obtained above) as light yellow plates with $[\alpha]_D^{25}$ −44 ± 2° (*c*, 1.23 in methanol) and m.p. 203–207° (dec.), the rotation of which was not altered by further recrystallization from ethanol.

Anal. Calcd. for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50. Found: C, 62.10; H, 6.68.

D-α-Acetamido-γ-p-nitrophenylbutyric acid (IIIb). To 52.2 g. of the D-α-acetamido-γ-p-nitrophenylbutyric acid (+)-α-phenylethylamine salt (0.135 mole) suspended in 300 ml. of water was added concentrated aqueous sodium hydroxide until the solid was completely dissolved and the (+)-α-phenylethylamine was completely separated from the aqueous solution. The amine was removed by washing with three 200-ml. portions of ether and, on acidification (*p*H 1) of the aqueous solution with concentrated hydrochloric acid, the organic acid precipitated. After cooling the mixture overnight at 0°, filtration, and recrystallization, there was obtained 33.3 g. of D-α-acetamido-γ-p-nitrophenylbutyric acid (92.8%) with properties as shown in Table I.

L-α-Acetamido-γ-p-nitrophenylbutyric acid (IIIb). In the same way as with its enantiomorph, 26.5 g. of the L-α-acetamido-γ-p-nitrophenylbutyric acid (−)-α-phenylethylamine salt (0.0684 mole) was decomposed with aqueous sodium hydroxide to 14.2 g. of L-α-acetamido-γ-p-nitrophenylbutyric acid (78.9%) with properties as shown in Table I.

Acknowledgment. We are indebted to Professor F. Bergel and Dr. J. A. Stock of the Chester Beatty Research Institute for Reference samples of the *p*-di-(β-chloroethyl)amino-DL-, -D-, and -L-phenylalanines and to Dr. C. L. Stevens of Wayne State University for providing the elemental analysis. This work was supported by a research grant-in-aid (CH-40) from the American Cancer Society to J. Murray Luck.

DETROIT, MICH.

[CONTRIBUTION FROM THE PHARMACEUTICAL LABORATORY, MEDICAL SCHOOL, KEIO-GIJUKU UNIVERSITY]

Santonin and Related Compounds. XVI.[1] C-Methylation of the Δ^4-3-Octalone Systems[2]

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The reaction of 9-methyl-Δ^4-3-octalone (I) with methyl iodide and potassium *tert*-butoxide readily yielded the 4,4,9-trimethyl ketone (II), as the chief product. In addition, 2,4,4,9-tetramethyl (III) and 4,9-dimethyl ketone (IV) were both obtained in minute amounts. Similar methylation of the dimethyl ketone (IV) proceeded much less readily leading to lower yield of the trimethyl ketone (II). A possible explanation is offered for these methylation reactions.

As described in the preceding paper of this series,[1] it became necessary to introduce one methyl group into the 4-position of Δ^4-3-octalone compounds. It had been previously reported[3] that the direct methylation of either Δ^4- or Δ^5-cholesten-3-one with

(1) Part XV, M. Yanagita, S. Inayama, M. Hirakura, and F. Seki, *J. Org. Chem.*, **23**, 690 (1958).

(2) This work was supported in part by the Grant in Aid for Scientific Research from the Japanese Ministry of Education.

(3) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954).