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# The Synthesis of Cyclic α-Amino Acids. I

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The reactions of α, β-unsaturated α-nitroesters with dienes produced cyclic α-nitroesters. In all cases, a mixture of diastereoisomers was obtained. Catalytic hydrogenation, followed by hydrolysis, gave a number of cyclic α-amino acids which are closely related to natural terpenes. The NMR spectra of some of these compounds were useful in making structural assignments.

As a part of the investigation of syntheses[1] from α, β-unsaturated α-nitroesters, their reactions as dienophiles have been studied. This study has shown that the Diels-Alder reaction between α, β-unsaturated α-nitroesters and appropriate dienes forms cyclic α-nitroesters, which can then be led to a new kind of cyclic α-amino acids by catalytic hydrogenation, followed by hydrolysis.

The Diels-Alder reaction of ethyl α-nitrocrotonate[1] (I) with cyclopentadiene under usual conditions gave ethyl 3-methyl-2-nitrobicyclo[2.2.1]-5-heptene-2-carboxylate (II). The catalytic hydrogenation of II with Raney Ni-T-4[2] gave ethyl 2-amino-3-methylbicyclo[2.2.1]heptane-2-carboxylate (III) together with a small amount of ethyl 2-hydroxyamino-3-methylbicyclo[2.2.1]heptane-2-carboxylate (IV). The catalytic hydrogenation of II with Adams' platinum oxide in acetic acid gave a dihydro derivative, *i. e.*, ethyl 3-methyl-2-nitrobicyclo[2.2.1]heptane-2-carboxylate (IX).

Although the hydrochloride of III gave correct analytical data, its melting point showed a wide range (171—188°C decomp.), suggesting that the amino ester obtained was a mixture of diasteroisomers. The saponification of III with barium hydroxide gave 2-amino-3-methylbicyclo[2.2.1]-heptane-2-carboxylic acid (V), which in turn gave acetyl and phenylhydantoin derivatives and copper salt. The amino acid (V) appeared as one spot on paper chromatograms. However, when it was oxidized with chloramine-T, a ketonic product was obtained; this product was identified by gas chromatography as a mixture of *exo*-apocamphenilone and *endo*-apocamphenilone. Thus, it was evident that the amino acid V is a mixture of diastereoisomers and that it has a bicyclo[2.2.1]-heptane ring.

The Diels-Alder reaction of I with butadiene gave ethyl 2-methyl-1-nitro-4-cyclohexene-1-carboxylate (VI). The gas chromatographic analysis

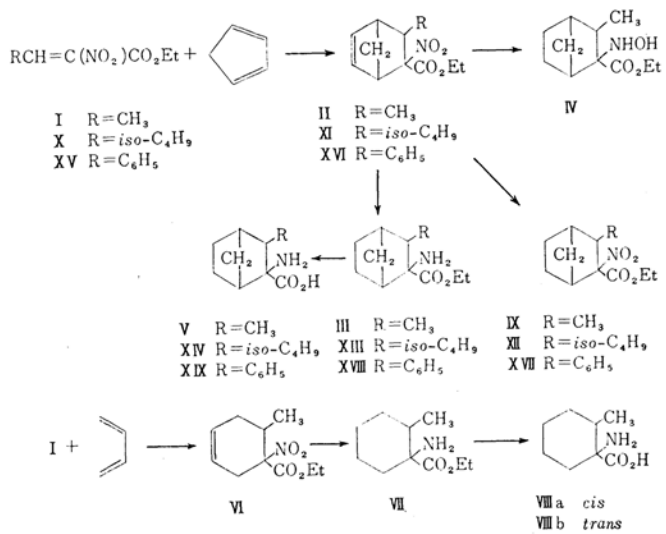


Chart 1

1) S. Umezawa and S. Zen, This Bulletin, **36**, 1143 (1963); *ibid.*, **36**, 1146 (1963); *ibid.*, **36**, 1150 (1963).

2) S. Nishimura, *ibid.*, **32**, 61 (1959).

of VI showed the presence of equal amounts of the two components. The catalytic hydrogenation of VI with Raney Ni-T-4 gave ethyl 1-amino-2-methylcyclohexane-1-carboxylate (VII). The saponification of VII with barium hydroxide gave 1-amino-2-methylcyclohexane-1-carboxylic acid (VIII). The paper chromatogram of VIII showed two spots, $R_f$ 0.59 and 0.53, which corresponded to the $R_f$ values of authentic 1-amino-*cis* and *trans*-2-methylcyclohexane-1-carboxylic acids[3] respectively. The *cis*-amino acid (VIIIa) was isolated from the ethanol-insoluble part of the mixture (VIII), while the *trans*-amino acid (VIIIb) was separated from the *cis*-isomer by cellulose-column chromatography. The IR spectra of these isomeric amino acids were in agreement with those of authentic samples.

Since the amino acid (VIII) consisted of two diastereoisomers (VIIIa and VIIIb), the starting material, I, was inferred to be a mixture of two geometrical isomers. I appeared as one peak on gas chromatograms which were obtained through a polyester-succinate column and a Reoplex 400 capillary column. Though the separation of the isomers was unsuccesful, the NMR spectrum of I in carbon tetrachloride showed that it was composed of two geometrical isomers. We observed a pair of overlapping quartets, centered at 2.80 and 3.12 $\tau$, which had the same coupling constant, $J$= 7.5 cps; they were assigned to the isomeric olefinic protons. Another pair of quartets, at 5.64 and 5.69 $\tau$, having the same $J$ value of 7.0 cps represented the ester methylene groups. A pair of doublets ($J$=7.0 cps) at 7.92 and 8.06 $\tau$ were characteristic of the $\beta$-methyl groups. The last pair of triplets ($J$=7.0 cps), at 8.63 and 8.67 $\tau$, could be attributable to the protons of ester methyl groups. The ratio of the amounts of the isomers was calculated as 1.8 : 1 on the basis of the integration of each peak area.

The adduct II was separated into two fractions, IIA and IIb, by gas chromatography. Both of these separated fractions were gas chromatographically pure, though their NMR spectra showed that IIA contained two diastereoisomers ($IIA_1$ and $IIA_2$), while IIB was a single diastereomer (Table 1). From IIA and IIB, the corresponding amino acids were derived; these amino acids were then oxidized with chloramine-T to the respective apocamphenilone. The ketone derived from IIA was a mixture of *exo*- and *endo*-apocamphenilone, while IIB gave only the *exo*-ketone.

The configurational relations between the three above-mentioned diastereoisomers ($IIA_1$, $IIA_2$ and IIB) in the adduct II were deduced by NMR spectroscopy. Fraser[4] observed that protons or proton-bearing substituents of bicyclo[2.2.1]-2-heptenes are shielded by the magnetically-anisotropic double bond in the *endo* 5-position, while they are deshielded in the *exo* 5-position. The saturation of the double bond removes the effect; the *endo* signals then move downfield while the *exo* signals move upfield. Kuivila[5] applied these

Table 1. Chemical shifts ($\tau$) of the fractions of adduct II and their hydrogenation products IX

| Compound | | 3-$CH_3$ (doublet) | Ester-$CH_2$- (quartet) | Ester-$CH_3$ (triplet) |
|---|---|---|---|---|
| IIA | $IIA_1$ | 8.97 | 5.82 | 8.73 |
| | $IIA_2$ | 9.09 | 5.84 | 8.75 |
| IIB | | 8.88 | 5.75 | 8.71 |
| IXA | $IXA_1$ | 9.10 | 5.76 | 8.73 |
| | $IXA_2$ | 9.02 | 5.82 | 9.75 |
| IXB | | 9.02 | 5.79 | 8.72 |

Ratio $IIA_1/IIA_2$=2 : 1

Table 2. Change in chemical shift ($\Delta\tau$) on hydrogenation

| Compound | 3-$CH_3$ | | Ester-$CH_2$ | |
|---|---|---|---|---|
| | *endo* | *exo* | *endo* | *exo* |
| $IIA_1 \rightarrow IXA_1$ | | +0.13 | −0.06 | |
| $IIA_2 \rightarrow IXA_2$ | −0.07 | | −0.02 | |
| $IIB \rightarrow IXB$ | | +0.14 | | +0.04 |

Table 3. Chemical shifts ($\tau$) of the fractions of dihydro derivative XII, adduct XVI and its dihydro derivative XVII

| Compound | | $-CH(CH_3)_2$ (doublet) | Ester methylene (quartet) | Ester methyl (triplet) |
|---|---|---|---|---|
| XIIA | | 9.07 | 5.79 | 8.73 |
| XIIB | $XIIB_1$ | 8.05 | 5.78 | 8.72 |
| | $XIIB_2$ | 9.06 | 5.82 | 8.75 |
| XVI | major | | 6.36 | 9.22 |
| | minor | | 5.57 | 8.72 |
| XVII | major | | 6.57 | 9.28 |
| | minor | | 5.67 | 8.65 |

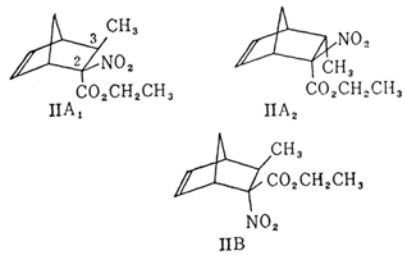


Fig. 1

3) R. J. W. Gemlyn, *J. Chem. Soc.*, **1962**, 3977; L. Munday, *ibid.*, **1961**, 4372.
4) R. R. Fraser, *Can. J. Chem.*, **40**, 78 (1962).
5) H. C. Kuivila and C. R. Warner, *J. Org. Chem.*, **29**, 2845 (1964).

TABLE 4. ADDACTS

| Compound | Reaction condition °C | hr | Mol-ratio* | Bp °C/3mmHg | $n_D$ (°C) | Formula | Found C, % | Found H, % | Found N, % | Calcd. C, % | Calcd. H, % | Calcd. N, % | Yield % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| II | 110 | 8 | 1.6 | 80—82 | 1.4780 (28) | $C_{11}H_{15}O_4N$ | 58.86 | 6.97 | 6.57 | 58.65 | 6.71 | 6.22 | 86 |
| VI | 130 | 6 | 16.4 | 60—90 | 1.4710 (22.5) | $C_{10}H_{15}O_4N$ | 56.08 | 7.06 | 6.77 | 56.32 | 7.09 | 6.57 | 49 |
| XI | 120 | 10 | 1.2 | 117—121 | 1.4799 (19) | $C_{14}H_{21}O_4N$ | 63.36 | 7.95 | 5.01 | 62.90 | 7.92 | 5.24 | 66 |
| XVI | 125 | 10 | 6.6 | 125—135 | 1.5432 (19) | $C_{18}H_{17}O_4N$ | 67.32 | 5.91 | 4.78 | 66.88 | 5.96 | 4.88 | 56 |

* Diene/dienophile

TABLE 5. GAS CHROMATOGRAPHIC ANALYSES

| Compound | Column length m | Column temp. °C | Injection temp. °C | Flow rate of helium ml/min | Rentention time min | |
|---|---|---|---|---|---|---|
| I | 2 | 168 | 180 | 45 | 11.7 | |
| X | 1 | 180 | 180 | 50 | 6.8 | |
| II | 2 | 168 | 180 | 45 | 33.2 | 38.8 |
| IIA | 2 | 168 | 180 | 45 | 33.2 | |
| IIB | 2 | 168 | 180 | 45 | | 38.8 |
| VI | 1 | 155 | 180 | 45 | 16.4 | 18.0 |
| XI | 1 | 190 | 210 | 50 | 14.9 | 16.4 |
| IX | 1 | 200 | 210 | 50 | 11.9 | 13.6 |
| XII | 1 | 200 | 210 | 50 | 16.0 | 18.5 |
| XIIA | 1 | 200 | 210 | 50 | 16.0 | |
| XIIB | 1 | 200 | 210 | 50 | | 18.5 |

observations to the configurational assignments of trimethylsilyl-substituted norbornenes. The chemical shifts of the protons of 3-methyl and 2-carbethoxy groups in IIA, IIB, IXA and IXB are given in Table 1. The changes n the chemical shift ($\Delta\tau$) of the protons in 3-methyl and 2-carbethoxy methylene groups on hydrogenation are shown in Table II. Following Fraser's observations, the configurations of the 3-methyl group and the 2-carbethoxy group in $IIA_1$, $IIA_2$ and IIB are assigned as shown in Fig. 1. The configurations of 3-methyl groups deduced by the NMR spectroscopy were verified by the chemical results obtained by the oxidation described above.

In addition, two other amino acids (XIV and XIX) of the above-mentioned type have been synthesized by an analogous route; the reactions of cyclopentadiene with ethyl 5-methyl-2-nitro-2-hexenoate (X) and ethyl α-nitrocinnamate[6] (XV) gave ethyl 3-isobutyl-2-nitrobicyclo[2.2.1]-5-heptene-2-carboxylate (XI) and ethyl 2-nitro-3-phenylbicyclo[2.2.1]-5-heptene-2-carboxylate (XVI) respectively. The partial hydrogenation of XI and XVI with Adams' platinum oxide gave ethyl 3-isobutyl-2-nitrobicyclo[2.2.1]heptane-2-carboxylate (XII) and ethyl 2-nitro-3-phenylbicyclo[2.2.1]heptane-2-carboxylate (XVIII) respectively. The complete hydrogenation of XI and XVI with Raney Ni-T-4 gave ethyl 2-amino-3-isobutylbicyclo-[2.2.1]heptane-2-carboxylate (XIII) and ethyl 2-amino-3-phenylbicyclo[2.2.1]heptane-2-carboxylate (XVIII), which, on hydrolysis with aqueous sodium hydroxide or barium hydroxide, afforded cyclic amino acids, *i. e.*, 2-amino-3-iso-butylbicyclo [2.2.1]heptane-2-carboxylic acid (XIV) and 2-amino-3-phenylbicyclo[2.2.1]heptane-2-carboxylic acid (XIX) respectively.

The NMR spectroscopic analysis showed that ethyl 5-methyl-2-nitro-2-hexenoate (X) contained two geometrical isomers, while the ethyl α-nitrocinnamate (XV) had a single isomer.

Gas chromatography revealed that the adduct XI was a mixture of diastereoisomers. The dihydro derivative, XII, could be separated into two fractions (XIIA and XIIB) by gas chromatography. The NMR spectra of XIIA and XIIB showed that XIIA was a single isomer, while XIIB consisted of two diastereoisomers, $XIIB_1$ and $XIIB_2$ (Table III).

The NMR spectra of the adduct XVI and its dihydro derivative, XVII, showed that both of them contained two diastereoisomers and that the configurations of the 2-carbethoxy groups of the major component were *exo*, and that of the minor component was *endo* (Table III). The oxidation of the amino acid XIX with chloramine-T yielded *exo*-phenylnorcamphor, which was detected by gas

6) A. Dornow and H. Menzel, *Ann.*, **588**, 40 (1954); A. Dornow, A. Müller and S. Lüpfert, *ibid.*, **594**, 191 (1955).

TABLE 6. DIHYDRO-DERIVATIVES OF ADDUCTS

| Compound | Bp °C/3 mmHg | $n_D^{21}$ | Formula | Found | | | Calcd. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | C, % | H, % | N, % | C, % | H, % | N, % |
| IX | 100—115 | 1.4784 | $C_{11}H_{17}O_4N$ | 58.28 | 7.53 | 6.06 | 58.13 | 7.54 | 6.16 |
| XII | 100—110 | 1.4737 | $C_{14}H_{23}O_4N$ | 62.77 | 8.30 | 5.13 | 62.43 | 8.61 | 5.20 |
| XVII | 140—150 | 1.5349 | $C_{16}H_{19}O_4N$ | 67.01 | 6.60 | 4.94 | 66.42 | 6.62 | 4.84 |

TABLE 7. CYCLIC $\alpha$-AMINO ESTERS

| Compound | Bp °C/mmHg | $n_D$ (°C) | Formula | Found | | | Calcd. | | | Yield % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C, % | H, % | N, % | C, % | H, % | N, % | |
| III | 40—55/0.01 | 1.4748 (33) | $C_{11}H_{19}O_2N$ | 66.65 | 9.78 | 7.00 | 66.97 | 9.68 | 7.10 | 75 |
| VII | 18—30/0.005 | 1.4677 (18) | $C_{10}H_{19}O_2N$ | 65.02 | 10.10 | 7.63 | 64.83 | 10.34 | 7.56 | 22 |
| XIII | 90—95/3 | 1.4755 (21) | $C_{14}H_{25}O_2N$ | 70.51 | 10.40 | 5.73 | 70.25 | 10.53 | 5.85 | 64 |
| XVIII | 100—115*/3 | 1.5350 (19) | $C_{18}H_{21}O_2N$ | 73.83 | 7.95 | 5.42 | 74.10 | 8.16 | 5.40 | 73 |

* Bath temperature

TABLE 8. HYDROCHLORIDES OF CYCLIC $\alpha$-AMINO ESTERS

| Compound | Mp °C | Solvent for recrystallization | Formula | Found | | | | Calcd. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C, % | H, % | N, % | Cl, % | C, % | H, % | N, % | Cl, % |
| III·HCl | 171—188 | Ethanol/ether | $C_{11}H_{20}O_2NCl$ | 56.65 | 8.63 | 6.26 | 15.28 | 56.52 | 8.62 | 5.99 | 15.17 |
| XIII·HCl | 161—163 | Ethanol/ether | $C_{14}H_{28}O_2NCl$ | 60.91 | 9.33 | 5.05 | 12.82 | 60.97 | 9.50 | 5.08 | 12.85 |

TABLE 9. CYCLIC $\alpha$-AMINO ACIDS AND THEIR HYDROCHLORIDES

| Compound | Mp °C | $R_f$-value | Formula | Found | | | | Calcd. | | | | Yield % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | C, % | H, % | N, % | Cl, % | C, % | H, % | N, % | Cl, % | |
| V | 298(decomp.) | 0.62 | $C_9H_{15}O_2N$ | 63.33 | 9.00 | 8.30 | | 63.88 | 8.94 | 8.28 | | 83 |
| XIV | 165—170 (sublim.) | 0.67 | $C_{12}H_{21}O_2N$ | 68.01 | 9.80 | 6.39 | | 68.21 | 10.02 | 6.63 | | — |
| XIV·HCl | 220(sublim.) | — | $C_{12}H_{22}O_2NCl$ | 58.24 | 8.78 | 5.59 | 14.36 | 58.17 | 8.95 | 5.65 | 14.31 | 64 |
| XIX·HCl | 200(sublim.) | 0.75 | $C_{14}H_{18}O_2NCl$ | 63.33 | 6.82 | 5.09 | 12.85 | 62.80 | 6.78 | 5.23 | 13.24 | 50 |

TABLE 10. *N*-ACETYL DERIVATIVES OF CYCLIC $\alpha$-AMINO ACIDS

| Compound from | Mp °C | Solvent for recrystallization | Formula | Found | | | Calcd. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | C, % | H, % | N, % | C, % | H, % | N, % |
| V | 208—223(decomp.) | Ethyl acetate | $C_{11}H_{17}O_3N$ | 62.85 | 8.15 | 6.51 | 62.54 | 8.11 | 6.63 |
| XIV | 209(sublim.) | Methanol/water | $C_{14}H_{23}O_3N$ | 66.41 | 8.84 | 5.54 | 66.37 | 9.15 | 5.33 |
| XIX | 236—237 | Methanol/water | $C_{18}H_{19}O_3N$ | 70.18 | 6.91 | 5.14 | 70.31 | 7.01 | 5.12 |

TABLE 11. COPPER SALTS OF CYCLIC $\alpha$-AMINO ACIDS

| Compound from | Solvent for recrystallization | Formula | Found | | | | Calcd. | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C, % | H, % | N, % | Cu, % | C, % | H, % | N, % | Cu, % |
| V | Methanol | $C_{18}H_{28}O_4N_2Cu\cdot H_2O$ | 51.98 | 7.73 | 6.63 | 15.58 | 51.72 | 7.21 | 6.70 | 15.20 |
| XIV | Methanol/water | $C_{24}H_{42}O_4N_2Cu$ | 59.50 | 8.22 | 5.71 | | 59.54 | 8.33 | 5.79 | |
| XIX | Methanol | $C_{28}H_{32}O_4N_2Cu$ | 63.59 | 6.12 | 5.54 | | 64.17 | 6.15 | 5.34 | |

chromatography and by the thin-layer chromatography of its 2, 4-dinitrophenylhydrazone derivative; no *endo*-isomer was detected.

## Experimental

Paper chromatograms of amino acids were run with *n*-butanol - acetic acid - water (4 : 1 : 1) on Toyo Roshi No. 50 papers using the ascending technique, and spots were detected by means of a spray of a 0.25% pyridine solution of ninhydrin.

Gas-liquid chromatographic analysis was mostly carried out with a Shimadzu-Kotaki Gas Chromatography, Model GU-21. The samples were chromatographed through a 4 mm i. d. copper column packed with polyester succinate on Shimalite. Analytical runs were made using 0.5 to 3 $\mu l$-samples, while for preparative runs 7- to 8-$\mu l$ samples were used.

The NMR spectra were obtained with a Varian A60 NMR spectrometer at a frequency of 60 Mc/sec. Each sample was run on a 10 or 20% (w/v) solution in carbon tetrachloride, containing 1% tetramethylsilane as an internal standard. The chemical shift values are considered to be accurate to $\pm 0.01$ ppm. The p$K_a$ values were determined by the method of Parke and Davis.[7]

**Ethyl 3-Methyl-2-nitrobicyclo[2. 2. 1]-5-heptene-2-carboxylate (II)** *(A Typical Example of the General Procedure).* A mixture of ethyl α-nitrocrotonate (I) (5.6 g, 0.035 mol), cyclopentadiene (3.7 g, 0.056 mol), and dry benzene (2.8 m*l*) was heated for 8 hr at 100—110°C in a sealed tube. The redbrown reaction mixture was then distilled in a vacuum. The main fraction was collected as a colorless oil boiling at 100—112°C/2 mmHg (yield 6.8 g (86%); $n_D^{25}$ 1.4795). Redistillation by means of the micro-method gave an analytical sample.

The other adducts, VI, XI and XVI, were prepared by the general procedure (Table 4). The results of gas chromatographic analyses of II, VI and XI are shown in Table 5.

**The Dihydro Derivatives of Adducts.** Adducts (*ca.* 500 mg) were dissolved in glacial acetic acid (5 m*l*) and hydrogenated with Adams' platinum oxide (50 mg) for 1 hr under atmospheric pressure. The products, IX, XII and XVII, are listed in Table 4. The results of the gas chromatographic analysis of IX and XII are given in Table 5.

**Ethyl 2-Amino-3-methylbicyclo[2. 2. 1]heptane-2-carboxylate (III)** *(A Typical Example of the General Procedure).* A solution of ethyl 3-methyl-2-nitrobicyclo-[2. 2. 1]-5-heptene-2-carboxylate (II) (2.64 g) in absolute ethanol (20 m*l*) was shaken with Raney nickel T-4[4] (4.3 m*l*) at 20—25°C and 120 kg/cm² for 4.5 hr in an autoclave. The contents were then filtered in order to remove the catalyst, and the solvent was distilled off in a vacuum. The ethereal solution was extracted with 1 N hydrochloric acid (9.8 m*l*) and washed with an aqueous, saturated, sodium chloride solution. The aqueous layer (pH 2.0) and washing were then combined and made alkaline by the addition of a 50% potassium carbonate solution. The separated oil was extracted with ether, and the ethereal extract was dried over potassium carbonate. After the evaporation of the solvent, the residue was subjected to vacuum distillation to give a colorless oil; yield, 1.72 g (75.2%); bp 50—58°C (bath temp.)/0.01 mmHg; $n_D^{25}$ 1.4790. Redistillation afforded an analytical sample of ethyl 2-amino-3-methylbicyclo[2. 2. 1]heptane-2-carboxylate (III). VII, XIII and XVIII were prepared by the general procedure (Table 7). The crystalline hydrochlorides of III and XIII were obtained from the ethanolic hydrogen chloride solution of III and XIII by dilution with absolute ether (Table 8).

**Ethyl 2-Hydroxyamino-3-methylbicyclo[2. 2. 1]-heptane-2-carboxylate (IV).** The ether layer remaining after the extraction of the amine ester (III) was again treated with 1 N hydrochloric acid. The acid extract was then made alkaline with a 50% potassium carbonate solution and extracted with ether. The dried etheral solution was evaporated in a vaccum to afford a colorless syrup which gradually crystallized; yield, 180 mg. (7%); mp 57—66°C. Redistillation gave a pure sample of IV, bp 60—90°C (bath temp.)/0.002 mmHg; mp 57—66°C. Tollens' test was positive. The sample showed a strong infrared absorption at $\nu_{max}^{KBr}$ 1104 cm$^{-1}$, indicating the presence of a hydroxyamino group.[8]

Found: C, 62.16; H, 9.07; N, 6.57%. Calcd for $C_{11}H_{19}O_3N$: C, 61.94; H, 8.98; 6.57%.

**The hydrolysis of Amine Esters.** The resulting amino acids and their derivatives are listed in Tables 9, 10 and 11.

**2-Amino-3-methylbicyclo[2. 2. 1]heptane-2-carboxylic Acid (V)** *(A Typical Example of the Gerneral Method for the Preparation of Free Amino Acid and Its Derivatives).* To a solution of the free amine ester (III) (1.25 g, 6.35 mmol) in methanol (11 m*l*), a 0.445 N barium hydroxide solution (15 m*l*, 3.33 mmol) was added. After the mixture had been allowed to stand for 20 hr at 36—37°C in an incubator, the reaction mixture was filtered to remove the insoluble material and then concentrated by evaporation. The residual aqueous solution was washed with ether twice and treated with 2 N sulfuric acid. The barium sulfate was removed by centrifuging. The supernatant layer (pH 5.8) and washings were then combined and evaporated in a vacuum. The residual colorless powder (0.99 g) was extracted with hot methanol (89 m*l*), and the extract was evaporated to dryness, yield, 0.89 g (83.1%). Recrystallization from methanol afforded an analytical sample of V, mp 298°C (decomp.); $R_f$ 0.62; p$K_{a_1}$ 2.3 and p$K_{a_2}$ 9.8.

*The N-Acetyl Derivative of V* was prepared by treating V with acetic anhydride in benzene.

*The Phenylhydantoin of V* was prepared according to the procedure of Cheronis[9]; mp 171—176.5°C (from methanol-water).

Found: C, 71.26; H, 6.52; N, 10.56%. Calcd for $C_{18}H_{18}O_2N_2$: C, 71.09: H, 6.71; N, 10.36%.

*Copper Salt of V* was prepared by treating V with copper acetate.

**The Separation and Identification of *cis*-and *trans*-1-Amino-2-methylcyclohexane-1-carboxylic Acid (VIII).** 1-Amino-2-methylcyclohexane-1-carboxylic acid (VIII) was prepared from VII by the procedure used in the preparation of V; the yield was

7) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).

8) R. T. Gilsdorf and F. F. Nord, *J. Am. Chem. Soc.*, **74**, 1837 (1952).

87.6%. The paper chromatogram of the product showed the presence of two components, $R_f$ 0.59 and 0.53 (purple with ninhydrin), which were in agreement with the $R_f$ values of the authentic 1-amino-*cis*-VIIIa and *trans*-2-methylcyclohexane-1-carboxylic acid (VIIIb) respectively.

The crude amino acid (VIII) (9.5 mg) was dissolved in boiling water and filtered to remove an insoluble material. The filtrate was evaporated to dryness, and the residue (6.1 mg) was washed with hot ethanol. The ethanol-insoluble product showed a single spot ($R_f$ 0.59) on a paper chromatogram and was identical in infrared spectrum (KBr) with the authentic *cis*-1-amino-2-methylcyclohexane-1-carboxylic acid (VIIIa) prepared by the Strecker method.[1]

The crude amino acid (VIII) (7 mg) was placed on a cellulose column (49×1.0 cm) and eluted with a *n*-butanol - acetic acid - water (4 : 1 : 1) solvent system; 0.5-m*l* fractions were then collected and examined by paper chromatography. Fractions 116—122 were combined and evaporated to dryness. After extraction with hot ethanol, the extract was concentrated and then diluted with ether to afford a crystalline powder. The product showed a single spot ($R_f$ 0.53) on paper chromatogram and was identical in infrared spectrum (KBr) with the authentic *trans*-1-amino-2-methylcyclohexane-1-carboxylic acid (VIIIb) prepared by the Bucherer method.[3]

**2-Amino-3-isobutylbicyclo[2. 2. 1]heptane-2-carboxylic Acid Hydrochloride (XIV·HCl)** *(A Typical Example of the General Method for the Preparation of Amino Acid Hydrochloride).* To a solution of the amine ester (XIII) (1.40 g) in methanol (8 m*l*), a 1 N sodium hydroxide solution (6.5 m*l*) was added; the mixture was then incubated for 20 hr at 37°C. After the mixture had then been evapolated to dryness, the residual powder was washed with ether and then dissolved in 3 N hydrochloric acid (6 m*l*). The acid solution was evaporated, and the residue was repeatedly extracted with ethanol. The evaporation of the extract gave a crude hydrochloride of XIV (1.72 g). Recrystallization from 80% aqueous acetic acid afforded colorless needles (0.93 g, 64%); mp 220°C (sublim.).

*Free Amino Acid XIV.* Prepared from the hydrochloride of XIV by treatment with 14% aqueous ammonia; after two sublimations, its melting point was 165—170°C (sublim.) and the $R_f$ value was 0.67.

*The N-Acetyl Derivative and Copper Salt of XIV* were prepared from XIV by the method described above (Tables 10 and 11).

**2-Amino-3-phenylbicyclo[2. 2. 1]heptane-2-carboxylic Acid Hydrochloride** (XIX·HCl) was prepared from XVIII by the procedure described above (Table 9). The *N*-acetyl derivative of XIX was prepared from the hydrochloride of XIX by treatment with fused sodium acetate and acetic anhydride in glacial acetic acid. Copper salt of XIX was prepared from the hydrochloride of XIX by treatment with sodium acetate and copper acetate.

**Chloramine-T Oxidation.** *The Oxidation of V.* A solution of chloramine-T (41.5 mg) in water (0.28 m*l*) was added to a solution of the amino acid (V) (21.3 mg) in water (0.36 m*l*), and the mixture was shaken for 20 min. The reaction mixture was then extracted with petroleum ether (bp 40—60°C), and the dried extract was evaporated. The residual colorless oil weighed 7.5 mg. Gas chromatographic analysis on a polyester-succinate column 2 m long at 151°C, with helium at 50 m*l*/min as the carrier gas, showed two peaks with the retention times of 6.4 min and 7.6 min. The retention times of these peaks were identical with those shown by authentic *exo*- and *endo*-apocamphenilone[10] respectively.

*The Oxidation of XIX.* After treating XIX with chloramine-T as described above, the reaction mixture was extracted with *n*-hexane and evaporated. The gas chromatographic analysis of the residue on a polyester succinate column 1 m long at 193°C, with helium at 60 m*l*/min, showed a peak with the retention time of 13.2 min; this peak was identical with that of authentic *exo*-3-phenylnorcamphor.[11] The treatment of the residue with 2, 4-dinitrophenylhydrazine, followed by purification by thin-layer chromatography on silica gel using the ethanol - *n*-heptane (1 : 4) solvent system, afforded a 2, 4-dinitrophenylhydrazone. Its $R_f$ value (0.70) was identical with that of an authentic sample derived from the *exo*-isomer.

**Ethyl 3-Hydroxy-5-methyl-2-nitrohexanoate.** Prepared from isovaleraldehyde and ethyl nitroacetate by the same method as reported by Umezawa and Zen; the yield was 61% and the boiling point was 120°C/3 mmHg, $n_D^{30}$ 1.4460.

Found: C, 49.66; H, 7.73; N, 6.69%. Calcd for $C_9H_{17}O_5N$: C, 49.31; H, 7.82; N, 6.39%.

**Ethyl 3-Acetoxy-5-methyl-2-nitrohexanoate.** Prepared by the acetylation of the above-mentioned ester; the yield was 87% and the boiling point was 140°C/3 mmHg, $n_D^{21.5}$ 1.4433.

Found: C, 50.78; H, 7.35; N, 5.51%. Calcd for $C_{11}H_{19}O_6N$: C, 50.56; H, 7.33, N, 5.36%.

**Ethyl 5-Methyl-2-nitro-2-hexenoate (X).** Prepared from the above-mentioned acetoxyester in a quantitative yield; bp 107—109°C/3 mmHg., $n_D^{24}$ 1.4564, $\lambda_{max}^{MeOH}$ 305 m$\mu$ ($\varepsilon$ 250); NMR signals were observed at $\tau$ ($CCl_4$): 2.87 and 3.23 (a pair of triplets, $\beta$-protons); 5.65 and 5.70 (a pair of quartets, ester methylene protons); 8.65 and 8.67 (a pair of triplets, ester methyl protons); 9.01 (a dublet, $\delta$-methyl protons).

Found: C, 53.84; H, 7.02; N, 6.85%. Calcd for $C_9H_{15}O_4N$: C, 53.72; H, 7.51; N, 6.96%.

*Ethyl α-Nitrocinnamate*[6] *(XV).* Nuclear magnetic resonance signals were observed at $\tau$($CCl_4$): 2.64 (phenyl and $\beta$-protons); 5.68 (a quartet, ester methylene protons); 8.66 (a triplet, ester methyl protons).

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