709. *The Chemistry of Extractives from Hardwoods. Part III.* Baikiain, an Amino-acid Present in* Baikiaea plurijuga.

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An amino-acid (baikiain), present chiefly in the water-soluble fraction of Rhodesian teak, the timber of *Baikiaea plurijuga*, has been shown to be L-1 : 2 : 3 : 6-tetrahydropyridine-2-carboxylic acid. Thus, on oxidation it gives a product from which trimethyl (*N*-carboxymethyl)-L-aspartate may be obtained, identical with a synthetic specimen.

The presence of baikiain among the pyrolysis products of L-1 : 2 : 3 : 6-tetrahydropyridine-2 : 4-dicarboxylic acid, as indicated by paper chromatography, supports the structure assigned on analytical evidence. The necessary tetrahydropyridinedicarboxylic acid was synthesised by well-known reactions from triethyl (*N*-carboxymethyl)-L-glutamate.

IN the course of a general investigation of the constituents of timbers stated to be resistant to decay and to the attack of wood-destroying insects, we have examined the heartwood of the Rhodesian tree, *Baikiaea plurijuga* (family, *Leguminosæ*). The timber is described commercially as Rhodesian teak, but it bears no botanical relationship to the well-known Burma teak, *Tectona grandis* (family, *Verbenaceæ*). Rhodesian teak is light reddish-brown, with characteristic irregular dark markings which impart a pleasing appearance to the finished material. The fine uniform grain gives it a smooth and very hard-wearing surface, and when imported into the United Kingdom it is thus chiefly used as a high-quality flooring timber.

The Rhodesian teak used in the following experiments was procured through the kindness of Mr. W. G. Campbell and Dr. W. P. K. Findlay, Forest Products Research Laboratory, Princes Risborough. Successive extractions of the powdered timber with light petroleum, ether, and chloroform yielded little product, but on treatment with cold ethanol 18% of the wood was recovered from the alcoholic extract as a dark reddish-brown powder, increased by percolation with hot alcohol to a total of 21—22%. Thereafter, extraction with hot water gave an additional 2—2½% of dark amorphous solid. Both the latter, and the alcoholic extract which also was readily soluble in water, were phenolic, and from their general properties appeared to consist largely of tannins; neither fractionation with solvents or as lead salts, nor the preparation of acetyl or benzoyl derivatives, etc., resulted in the isolation of any homogeneous product. However, an aqueous solution of the combined alcohol and water-soluble fractions, from which tannin substances had been completely removed with lead acetate, after suitable manipulation yielded a minute quantity of crystalline solid, m. p. 274°, dissolving somewhat sparingly in ethanol but freely soluble in water. A water extraction of the timber was therefore made omitting pretreatment with alcohol, and by dissolving the resulting solid in methanol and concentrating to a certain critical volume, appreciable amounts of the new product, subsequently termed *baikiain*, were successfully crystallised.

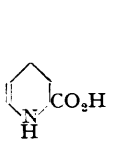
A simplified process of isolation affording much larger yields was then devised, depending on the deposition of the tannin content as reddish-black resinous solid on heating the extract with mineral acid. Incidentally, the modified extraction technique revealed the basic character of the new compound. At first, the total water-soluble extract of the timber was so treated, but later it proved sufficient simply to stir the powdered wood, without any previous solvent treatment, in cold dilute hydrochloric acid, whereupon, although only a relatively small proportion of tannin was extracted, isolation of the baikiain was not adversely affected. The aqueous solution was afterwards concentrated to small bulk by being heated on a steam-bath, and separated from the resinous deposit. Evaporation to dryness then left a semi-solid residue which when triturated with cold methanol gave a colourless crystalline solid (1 g./100 g. timber) consisting of baikiain hydrochloride. To determine whether this considerably larger yield might be due to the existence in the wood of some compound of baikiain sensitive to acid hydrolysis, a separation was carried out in which the phenolic constituents of a cold water extract were eliminated by shaking it with activated alumina. The isolation from the remaining colourless neutral solution of some 80% of the amount previously obtained by using the hydrochloric acid method shows that the baikiain content of the wood exists substantially uncombined.

The solubility characteristics and high melting point of baikiain, and the presence of nitrogen, were suggestive of an amino-acid structure, and in the early stages, before analyses had been completed, attempts were made to identify the small quantity then available by partition chromatography, phenol saturated with water being used as the mobile phase. A comparatively

* Recent publications entitled "The Constitution of Chlorophorin, etc." (F. E. King and M. F. Grundon, *J.*, 1949, 3348; 1950, 3547), are considered to be Parts I and II of this series.

high R_F value of 0·82 was found, and this, together with the yellow-brown ninhydrin colour, which is characteristic of the restricted group of cyclic α-amino-acids such as proline, was sufficient to show that the compound was not one of the known amino-acids. This became obvious with the completion of analyses of baikiain, its hydrochloride, picrate, *N*-benzoyl derivative, methyl ester, methyl ester hydrochloride, and *N*-benzoyl methyl ester, all of which corresponded to an empirical formula of $C_6H_9O_2N$. The low boiling point of the methyl ester left little doubt that this was also the molecular formula of the amino-acid.

Catalytic reduction of the amino-acid and of its hydrochloride resulted in the addition of one molecule of hydrogen, thus limiting the constitution to that of a methylpyrroline- or tetrahydropyridine-carboxylic acid. The nature of the heterocyclic structure, and also the position of the substituent carboxyl group, was shown by heating the hydrochloride with zinc dust whereupon an oil having a strong pyridine odour was collected. A comparison of its picrate with those of the three picolines proved the distillate to be α-picoline. Complete reduction of the carboxyl group in the tetrahydropyridine acids during zinc-dust distillation appears to be characteristic of the series; guvacine (1 : 2 : 5 : 6-tetrahydropyridine-3-carboxylic acid) under these conditions forms β-picoline (Jahns, *Arch. Pharm.*, 1891, **229**, 693; Freudenberg, *Ber.*, 1918, **51**, 1675).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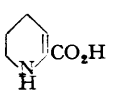
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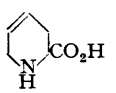
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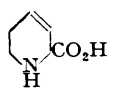
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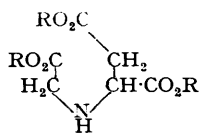

(V.)

$HO_2C{\cdot}[CH_2]_2{\cdot}NH{\cdot}CH{\cdot}(CO_2H)_2$
(VI.)

$HO_2C{\cdot}[CH_2]_2{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$
(VII.)

Baikiain has in aqueous solution a strong negative specific rotation. The dihydro-derivative formed by catalytic reduction appears from the scanty published data to be identical with the lævo-rotatory isomer of pipecolinic acid. For dihydrobaikiain, however, a value $[\alpha]_D^{23} = -25{\cdot}2°$ was found, whereas Mende (*Ber.*, 1896, **29**, 2889) has given $[\alpha]_D^{25} = -34{\cdot}9°$ for a synthetical specimen resolved *via* the tartrate. On the other hand, the carefully purified acid obtained by Willstätter (*Ber.*, 1901, **34**, 3168) from the oxidation of conhydrine has $[\alpha]_D^{24} = -24{\cdot}7°$ which is in good agreement with our measurement.

Having established that baikiain is a tetrahydropicolinic acid, it remained to ascertain the situation of the double link. The Δ^1- and Δ^6-positions are possibilities that can be dismissed immediately, not merely because baikiain forms an *N*-benzoyl derivative, which could be explained on the basis of tautomerism, but because the acid stability of the compound is incompatible with a Schiff's base structure. The absence of any decomposition or polymerisation on rigorous treatment with acid or alkali may be taken to exclude (I) which possesses a vinylamine group and although this consideration may have less significance in (II) where some stabilisation is to be expected from the proximity of the carboxyl group, this latter formulation is inadmissible since the optical activity of baikiain requires the presence of an asymmetric centre. Of the remaining two structures, (III) seemed to be the more likely because (IV), being a βγ-unsaturated acid, would undergo comparatively easy racemisation, whereas the amino-acid suffered only partial loss of optical activity after 12 hours in boiling 40% aqueous sodium hydroxide.

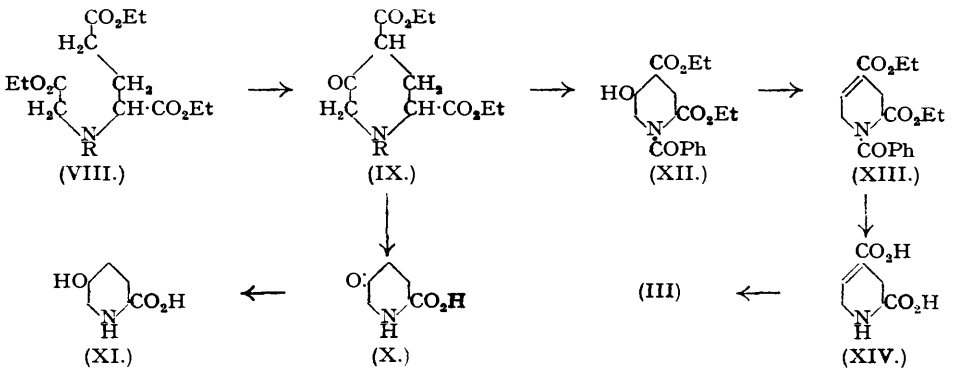
The Δ^4-structure (III) implies that under suitable conditions baikiain would be oxidised to the imino-tricarboxylic acid (V; R = H), but before this degradation was attempted a synthesis was undertaken of the expected product in the form of its trimethyl ester, believed to be the most suitable for the preparation of easily recognisable crystalline derivatives. In order that there should be no uncertainty in identifying the oxidation product, methyl esters of the acids to be expected from the alternative though less probable structure (IV) were also synthesised, that is, (VI) and its possible decarboxylation product (VII). The Δ^5-compound (I) would have been oxidised ultimately to L-glutamic acid; the hitherto unreported picrolonate of its dimethyl ester was prepared in the course of this investigation.

The appropriate optically-active trimethyl ester of *N*-carboxymethylaspartic acid was prepared from methyl L-aspartate and methyl chloroacetate, and the esters of (VI) and (VII), respectively, by the action of methyl bromomalonate and bromoacetate on β-aminopropionitrile

followed by methanolysis of the products. Each of these esters was characterised by a crystalline picrate and picrolonate.

Several attempts were made to oxidise baikiain and its compounds with ozone and with potassium permanganate before the necessary conditions were found. The methyl ester and *N*-benzoylbaikiain gave only insignificant amounts of impure material, but ozonolysis of *N*-benzoylbaikiain methyl ester was partly successful. However, the most consistent and satisfactory results were obtained from the action of ozone on a solution of baikiain in dilute hydrochloric acid, the oxidation being completed with hydrogen peroxide. Appreciable resinification invariably accompanied the final stages, but after evaporation, and esterification of the dark residue, it was possible by distillation to obtain an oil giving a picrate and picrolonate identical with those of the ester (V; R = Me), thus firmly establishing the constitution attributed to baikiain on general grounds. Correspondence between the specific rotation of the esters from natural and synthetic sources gave further support to this conclusion as well as confirming that the cyclic amino-acid is a member of the usual L-series. The characteristic lowering of the specific rotation on salt formation was also observed both with baikiain and with its dihydro-derivative and their hydrochlorides.

Experiments on the synthesis of baikiain followed the course indicated below and were based on L-glutamic acid in order that optical resolution of the final product would not be necessary:



Triethyl *N*-carboxymethyl-L-glutamate (VIII; R = H) was readily prepared from ethyl L-glutamate and ethyl bromoacetate in acetone in presence of potassium carbonate. Cyclisation to a piperidone was first carried out with the acetyl derivative, but better results were obtained with the *N*-benzoyl compound (VIII; R = COPh), as in an analogous ring-closure described by McElvain and Stork (*J. Amer. Chem. Soc.*, 1946, **68**, 1049), and treatment with powdered sodium in benzene gave an oily product which, although it may have contained appreciable amounts of the isomeric 2 : 6-dicarboxylate, from the results of later reactions appears to have consisted largely of (IX; R = COPh). Hydrolysis in boiling hydrochloric acid gave 5-keto-piperidine-2-carboxylic acid (X) which was obtained as a crystalline hydrochloride; a 2 : 4-dinitrophenylhydrazone hydrochloride was also prepared. Catalytic reduction of the hydrochloride of (X), despite the generation of a new asymmetric centre, produced a homogeneous product, but in its reactions with the standard dehydrating agents the resulting 5-hydroxy-piperidine-2-carboxylic acid (XI) gave no evidence of the formation of either the desired tetrahydropyridine-2-carboxylic acid or even of its Δ^5-isomeride.

The projected synthesis was therefore revised, the diethyl 1-benzoyl-5-ketopiperidine-2 : 4-dicarboxylate (IX; R = COPh) being first reduced to the hydroxy-diester (XII), and dehydration attempted before decarboxylation at the 4-position. The glassy product obtained by catalytic reduction of (IX; R = COPh) was heated to 180° in a stream of hydrogen chloride (cf. McElvain and Stork, *loc. cit.*), and without isolation of the intermediate, it was hydrolysed with boiling hydrochloric acid to give the hydrochloride of the tetrahydropyridinedicarboxylic acid (XIV). However, better results were obtained when the carbinol (XII) was dehydrated in pyridine or dimethylaniline with thionyl chloride. The less viscous product, presumably (XIII), was then hydrolysed with hydrochloric acid. From the resulting crystalline salt, 1 : 2 : 3 : 6-tetrahydropyridine-2 : 4-dicarboxylic acid (XIV) was liberated by treatment with silver carbonate, but the 4-carboxyl group, which was expected to be the more labile because of its attachment to unsaturated carbon, could not be eliminated by the ordinary methods of decarboxylation.

Finally, (XIV) was heated under pressure with concentrated hydrochloric acid, as in the conversion of anhydroecgonine into tropidine, but the residue was an intractable tar. Experience had shown, however, that both the natural amino-acid and its hydrochloride could be successfully sublimed without even the loss of optical activity, and so the product was heated at low pressure. The colourless sublimate contained a high proportion of ammonium chloride thus preventing the isolation of the organic substance. The solid was therefore dissolved in water and the synthetic amino-acid separated from the contaminant by partition chromatography side by side with an aqueous solution of baikiain hydrochloride. The appearance on development with ninhydrin of a stain indistinguishable in its colour and position from the natural amino-acid seemed clearly to show the presence of the identical compound in the synthetic product.

The method of purification was then applied to the material obtained when the free dicarboxylic amino-acid (XIV) was heated alone or with copper powder, but the quantity of distillate was smaller and it required resublimation before the presence of baikiain could be shown by the chromatographic method.

Experimental.

Baikiain.—(i) The evaporated aqueous extract (5·0 g.) from powdered Rhodesian teak (250 g.), previously extracted with ethanol, was treated for 2 hours with boiling methanol in a Soxhlet apparatus. The solution was then concentrated to 20 c.c., and on being left overnight at 0° it deposited baikiain (0·6 g.) as a nearly colourless crystalline powder.

(ii) The powdered wood (2 kg.) was vigorously stirred at room temperature with dilute hydrochloric acid (3 l.; 2N.) for 6 hours. The liquid obtained by filtration was evaporated under slightly reduced pressure on a steam-bath to 300 c.c., the precipitated phlobaphene removed, and the filtrate, after treatment with charcoal, evaporated to dryness. The brown semi-solid mass thus obtained gave baikiain hydrochloride (20—24 g.) on trituration with methanol. Evaporation of the methanol residue after charcoal treatment gave a gummy material (20 g.) which has failed to give any further crystalline substance.

(iii) The powdered wood (500 g.) was warmed with water (1 l.) at 70° for 3 hours, and after being separated as far as possible from the resulting solution it was again extracted under the same conditions. The combined extracts were concentrated by evaporation at 50 mm., and the red solution (1 l.) treated with successive quantities of activated alumina until it was practically colourless. Evaporation of the solution at low pressure left a semi-solid residue which gave crystalline baikiain (3·0 g.) on trituration with methanol; an uncrystallisable gummy product (5·0 g.) was obtained from the methanol.

Baikiain crystallises from methanol in thick glistening prisms, m. p. 274° (decomp.) (Found: C, 56·6; H, 7·2; N, 10·9. $C_6H_9O_2N$ requires C, 56·7; H, 7·1; N, 11·0%). It is very soluble in water but only sparingly soluble in hot or cold ethanol and insoluble in acetone, ether, benzene, and ethyl acetate. By chromatographic analysis, using the paper-strip technique with phenol saturated with water as the mobile phase and glycine, valine, and phenylalanine as standards, baikiain was shown to have an R_F value of 0·82. The colour produced on development with ninhydrin was yellow-brown. The optical rotatory power of a solution of 0·989 g. in 10 c.c. of water in a decimetre tube was −19·85°, giving $[\alpha]_D^{20} = -201·6°$.

Baikiain hydrochloride obtained as by method (ii) is very soluble in water, and rather more soluble in methanol and particularly ethanol than is the free base; it is insoluble in acetone, ether, benzene, and ethyl acetate. Crystallised from methanol or ethanol it formed glistening flat-ended prisms, m. p. 264° (decomp.), $[\alpha]_D^{20}$, in aqueous solution, = −90·1° (Found: C, 44·2; H, 6·4; N, 8·5. $C_6H_9O_2N,HCl$ requires C, 44·0; H, 6·2; N, 8·6%).

The *picrate* was formed on addition of baikiain hydrochloride to saturated aqueous sodium picrate. It was quite soluble in cold water and very soluble in hot water from which it separated as yellow needles or plates, m. p. 172—173° (Found: C, 40·6; H, 3·4. $C_6H_9O_2N,C_6H_3O_7N_3$ requires C, 40·5; H, 3·4%).

Baikiain was quite unchanged by being boiled with concentrated hydrochloric acid for 6 hours. The action of boiling 40% aqueous sodium hydroxide was followed polarimetrically. Racemisation was very slow, being incomplete after 12 hour's heating. No attempt was made to recover any products from this reaction.

N-*Benzoylbaikiain.*—Baikiain hydrochloride (2·0 g., 1 mol.) in aqueous sodium hydroxide (25 c.c.; 2N., 4 mol.) was vigorously shaken with benzoyl chloride (2·1 g., 1·2 mols.). When the acid chloride had disappeared, the solution was filtered from a small amount of flocculent material and acidified (Congo-red) with hydrochloric acid. *Benzoylbaikiain* rapidly separated and solidified, and crystallised from ethyl acetate as prisms (2·0 g., 71%), m. p. 178—179°, $[\alpha]_D^{20} = -91·9°$ (Found: C, 67·8; H, 5·35; N, 6·2. $C_{13}H_{13}O_3N$ requires C, 67·5; H, 5·65; N, 6·1%). It also dissolved in ethanol and acetone, but was very sparingly soluble in ether and in water from which it crystallised in needles.

Attempts to acetylate baikiain with acetic anhydride were unsuccessful; a vigorous reaction occurred on heating which gave brown resinous products.

Baikiain Methyl Ester.—Baikiain hydrochloride (2·0 g.), dissolved in anhydrous methanol (50 c.c.) saturated with hydrogen chloride, was heated under reflux for 5 hours. The solution was then evaporated under reduced pressure and the gummy residue triturated with acetone, giving the methyl ester *hydro-*

chloride as a colourless crystalline solid (1·9 g., 87·5%), m. p. 158° (decomp.). Crystallisation by addition of an equal volume of acetone to its saturated solution in methanol gave long prisms, m. p. 164° (decomp.) (Found: C, 46·9; H, 6·9; N, 8·3. $C_7H_{11}O_2N$,HCl requires C, 47·3; H, 6·8; N, 7·9%).

The ester hydrochloride (0·95 g.) was suspended in dry benzene (15 c.c.) and anhydrous diethylamine (2 c.c.) added. After 2 hours' shaking, dry ether (30 c.c.) was added and the precipitated solid removed. Fractionation of the filtrate gave the *ester* as a basic-smelling oil (0·45 g., 59%), b. p. 110—112°/15 mm. (Found: C, 59·1; H, 8·3. $C_7H_{11}O_2N$ requires C, 59·55; H, 7·9%). The picrate of the base was oily. The benzoyl derivative (yield 63%) was obtained by heating the ester hydrochloride in boiling toluene with benzoyl chloride, but it was more readily prepared by treating an ethereal suspension of benzoylbaikiain with excess of diazomethane. It is a viscous oil, b. p. 140—145° (air-bath temp.)/0·1 mm., rapidly turning brown in air, and very readily hydrolysed, liberating benzoic acid (Found: N, 6·0. $C_{14}H_{15}O_3N$ requires N, 5·7%).

Baikiain Hydrochloride. Zinc Dust Distillation.—The amino-acid hydrochloride (0·1 g.) was mixed with zinc dust (1·0 g.), and the mixture heated. The evil-smelling distillate, collected in ethanol, was treated with excess of saturated alcoholic picric acid, giving a copious precipitate (0·1 g., 50%), m. p. 156—159° raised by two crystallisations (charcoal) from ethanol to 163°. For α-picoline picrate several values are given in the literature ranging from 163° to 169—171°; we found for the picrate prepared from pure α-picoline, m. p. 163°. The salt obtained from the degradation product was identical in solubility and appearance with the authentic specimen, and the mixed m. p. was 163° (Found: N, 17·5. Calc. for C_6H_7N,$C_6H_3O_7N_3$: N, 17·4%).

Dihydrobaikiain (L-*Pipecolinic Acid*).—(i) Baikiain hydrochloride (1 g.) in methanol (60 c.c.) containing concentrated hydrochloric acid (0·5 c.c.) was shaken at atmospheric pressure and temperature with hydrogen in the presence of platinum (from Adams's platinic oxide, 0·05 g.). Uptake of hydrogen was complete in 20 minutes, and 135 c.c. had been absorbed (calc. 137 c.c.). The filtered solution was evaporated to a small bulk and treated with acetone. The crystalline precipitate, crystallised from ethanol, then had m. p. 256° (decomp.), $[\alpha]_D^{18} = -5{\cdot}0°$ (Found: C, 43·8; H, 7·4. Calc. for $C_6H_{11}O_2N$,HCl: C, 43·5; H, 7·2%). Willstätter (*loc. cit.*) gives m. p. 256—258° for (−)-pipecolinic acid hydrochloride.

(ii) Baikiain (1 g.) in methanol (90 c.c.) was hydrogenated with Adams's platinum catalyst at room temperature and pressure. Uptake of hydrogen (174 c.c.; calc. 177 c.c.) was complete in 1 hour. Removal of catalyst and evaporation of the solution to dryness gave a solid giving prisms (0·65 g.), m. p. 268° (decomp.), $[\alpha]_D^{18} = -25{\cdot}2°$, on crystallisation from methanol. Mende (*loc. cit.*) gives for (−)-pipecolinic acid, m. p. 270° (decomp.), $[\alpha]_D^{25} = -34{\cdot}9°$; Willstätter (*loc. cit.*), m. p. 264—265°, $[\alpha]_D^{24} = -24{\cdot}7°$. Both the amino-acid and its hydrochloride readily sublimed below 15 mm. without decomposition.

N-*Benzoyldihydrobaikiain.*—(i) *N*-Benzoylbaikiain (0·8 g.) was hydrogenated by using Adams's catalyst in methanol at room temperature and pressure. After 45 minutes the reaction ceased (uptake 75 c.c.; calc. 77 c.c.), and after removal of catalyst the solvent was evaporated and the residue crystallised from water, forming N-*benzoyldihydrobaikiain* as long needles (0·6 g., 75%), m. p. 145°, $[\alpha]_D^{18}$ −72·8° (Found: C, 66·4; H, 6·1; N, 5·9. $C_{13}H_{15}O_3N$ requires C, 66·9; H, 6·4; N, 6·0%).

(ii) Dihydrobaikiain (0·5 g.) dissolved in dilute sodium hydroxide (10 c.c., 2N.) was shaken with benzoyl chloride (0·55 g., 1·25 mols.). When the acid chloride had disappeared, the solution was acidified and the oily precipitate induced to crystallise by seeding with material from (i) above, giving the colourless derivative, m. p. 145° after one crystallisation from water.

Trimethyl N-2-*Carboxyethylaminomalonate.*—β-Aminopropionitrile (*Org. Synth.*, 1947, **27**, 3) (13·2 g., 2 mols.) was treated with methyl bromomalonate (19 g., 1 mol.), and the very vigorous reaction moderated by cooling under the tap. When the reaction had subsided, the semi-solid mass was heated for 2 hours on the steam-bath, dissolved in methanol (120 c.c.), and saturated with hydrogen chloride. After 5½ hours' boiling, separation of ammonium chloride ceased, and the solid was removed and the filtrate evaporated on a steam-bath under reduced pressure. Water (200 c.c.) was added to the residue and insoluble matter removed with chloroform; the aqueous layer was separated, basified strongly with solid potassium carbonate, and extracted four times with ether. The dried extract (Na_2SO_4) on distillation gave the *triester* as a pale-yellow viscous liquid (10·0 g., 45·5%), b. p. 110—112°/0·1 mm. (Found: N, 6·2. $C_9H_{15}O_6N$ requires N, 6·0%).

The picrate, prepared in dry ether, separated as an oil. A portion in one experiment crystallised, on being kept at 0°, in large prisms, m. p. 111—112°, but subsequent attempts failed to yield crystalline material again.

The *picrolonate* was prepared in methanol, from which it separated after 2 weeks at 0° as clumps of short prisms, which after recrystallisation from methanol had m. p. 131—132° (Found: C, 45·8; H, 4·5. $C_9H_{15}O_6N$,$C_{10}H_8O_5N_4$ requires C, 45·9; H, 4·7%).

Dimethyl β-(N-*Carboxymethyl*)*aminopropionate.*—A solution of β-aminopropionitrile (10 g., 1 mol.) and methyl chloroacetate (16 g., 1 mol.) in benzene (100 c.c.) was heated to boiling for 2½ hours. The solvent was then evaporated on a steam-bath and the residue dissolved in methanol (100 c.c.) saturated with hydrogen chloride. After 9 hours' refluxing, ammonium chloride was removed and the solution evaporated, the product being isolated by shaking the residue with aqueous sodium hydroxide (50 c.c.; 20%) and chloroform. Distillation of the dried chloroform solution gave the *dimethyl* ester of (VII), b. p. 110—112°/3 mm. (Found: C, 47·7; H, 7·7. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·4%). The *picrate*, prepared in anhydrous ether, separated as a gum which solidified after several days at 0°. Recrystallisation from methanol–ether or a small volume of methanol gave yellow needles or prisms, m. p. 113° (Found: N, 13·9. $C_7H_{13}O_4N$,$C_6H_3O_7N_3$ requires N, 13·95%). The sparingly soluble

picrolonate crystallised from methyl alcohol in bright-yellow fern-like masses, m. p. 143—144° (Found : C, 46·3; H, 4·8. $C_7H_{13}O_4N,C_{10}H_8O_5N_4$ requires C, 46·45; H, 4·8%).

Methyl Glutamate.—The *picrolonate* of the ester prepared from glutamic acid by standard methods crystallised from methanol in thick, yellow leaflets, m. p. 168° (Found : C, 46·6; H, 4·9; N, 15·4. $C_7H_{13}O_4N,C_{10}H_8O_5N_4$ requires C, 46·5; H, 4·8; N, 15·9%). The picrate was oily.

Trimethyl N-*Carboxymethyl*-L-*aspartate* (V; R = H).—When methyl aspartate (3·8 g., 2 mols.) and methyl bromoacetate (1·8 g., 1 mol.) were mixed the temperature rose slightly. After 12 hours, the dark red solution was poured into dilute hydrochloric acid (50 c.c.; 2N.) and the acid-insoluble material was removed with ether; excess of potassium carbonate was then added to the aqueous layer, and the oily precipitate extracted thrice with ether. The dried (Na_2SO_4) extract on distillation gave the *triester* (1·4 g., 51%) as an oil, b. p. 120° (air-bath temp.)/0·1 mm., $[\alpha]_D^{20} = -11·9°$ (Found : C, 46·5; H, 6·5. $C_9H_{15}O_6N$ requires C, 46·3; H, 6·5%).

The *picrate* was prepared by mixing ethereal solutions of the ester and picric acid. A slightly turbid solution was thus obtained which deposited crystals on being left overnight at 0°. Recrystallisation was accomplished by dissolution in a very little hot methanol and adding ten times the volume of dry ether; after a while the salt crystallised in pale-yellow leaflets, m. p. 137° (Found : C, 39·1; H, 3·6; N, 12·2. $C_9H_{15}O_6N,C_6H_3O_7N_3$ requires C, 39·0; H, 3·9; N, 12·1%).

The *picrolonate* was prepared in methanol, from which it separated slowly in long silky needles, and after recrystallisation had m. p. 182° (Found : C, 46·2; H, 5·0. $C_9H_{15}O_6N,C_{10}H_8O_5N_4$ requires C, 45·9; H, 4·7%).

Oxidation of Baikiain.—Excess of ozone was passed into a solution of baikiain hydrochloride (2·0 g.) in dilute hydrochloric acid (10 c.c.; 1%) at 0°. Afterwards, hydrogen peroxide (5 c.c.; 40 vol.) was added, and 3 hours later a few mg. of platinum black, and the whole left overnight. The resulting pale-yellow solution, after removal of the platinum, was evaporated to dryness at −5°/0·1 mm.; during the last stages of the evaporation the residue became very black. Methanol (50 c.c.) was added to the residue, and the solution saturated with hydrogen chloride and boiled for 4 hours. After evaporation nearly to dryness in a vacuum the residue was dissolved in water (40 c.c.), saturated with potassium carbonate, and extracted four times with chloroform (total 100 c.c.). Distillation of the extract gave an oil (150—250 mg., 5—8%), b. p. 120° (air-bath temp.)/0·1 mm.

The quantity of trimethyl (*N*-carboxymethyl)-L-aspartate present in the distillate varied, but in three experiments conversion into the crystalline picrate indicated yields of 80%; for one of the samples of ester was found $[\alpha]_D^{20} = -9·5°$. The picrate had m. p. 137° alone or mixed with the synthetic trimethyl (*N*-carboxymethyl)-L-aspartate picrate. Similarly the picrolonate, m. p. 182°, was indistinguishable from that prepared from the synthetic ester (V; R = Me).

N-Benzoylbaikiain methyl ester (2·8 g.) in dry ethyl acetate (20 c.c.) was treated with excess of ozone at 0°. The solution was evaporated to dryness at 30° to give a cream-coloured resin which was oxidatively decomposed by cold 5% aqueous potassium permanganate added dropwise until decolorisation was slow. After removal and washing of the manganese dioxide, the brown solution was acidified with sulphur dioxide and extracted four times with ether (total 200 c.c.). Evaporation of the solvent gave an oil which was hydrolysed by boiling it with hydrochloric acid (100 c.c.; 15%) for 2 hours. On cooling, benzoic acid separated and was removed, and the solution was evaporated to dryness. Esterification and further treatment as above gave an oil (200 mg., 7%), b. p. 120° (air-bath temp.)/0·1 mm., yielding the picrate and picrolonate previously obtained. The purity of the ester thus prepared was inferior to that obtained from baikiain hydrochloride, and the two derivatives were correspondingly more difficult to purify.

Triethyl N-*Carboxymethyl*-L-*glutamate* (VIII; R = H).—Ethyl glutamate hydrochloride (from 25 g. of glutamic acid) was dissolved in water (100 c.c.) saturated with potassium carbonate and the mixture extracted 10 times with ether. The crude ethyl glutamate (19 g., 1 mol.) obtained by evaporating the dried extracts was mixed with acetone (60 c.c.) containing anhydrous potassium carbonate (13·2 g., 1 mol.) and treated with ethyl bromoacetate (16·8 g., 11 c.c., 1·05 mols.). The temperature of the vigorously shaken mixture rose slowly during 1 hour to 35°, and then fell, and after 12 hours at room temperature the solid material was collected and well washed with ether. The filtrate and washings were evaporated on the steam-bath under reduced pressure, water (100 c.c.) was added to the residue, and the solution made acid (Congo-red). The small quantity of acid-insoluble material was removed with ether, and the aqueous layer basified with an excess of potassium carbonate. An oil separated which was taken into ether and dried (Na_2SO_4). Distillation at reduced pressure gave a pale-yellow oil, b. p. 150—155°/0·4 mm. (18·5 g., 68% based on ethyl glutamate), but satisfactory analyses could not be obtained and subsequent experiments indicated the presence of non-basic material, presumably the corresponding pyrrolidone formed by elimination of alcohol. When the distilled ester (8·4 g.) was dissolved in excess of acetic anhydride (10 g.), the temperature rose to about 40°, and after 2 hours on a steam-bath the liquid was distilled, giving the *acetyl* compound (VIII; R = COMe) (7·6 g., 79%), b. p. 145—150°/0·1 mm., as a viscous pale-yellow oil (Found : C, 53·9; H, 7·4; N, 5·0. $C_{15}H_{25}O_7N$ requires C, 54·4; H, 7·6; N, 4·2%).

Diethyl 1-*Acetyl*-5-*ketopiperidine*-2 : 4-*dicarboxylate* (IX; R = COMe).—The acetyl derivative (VIII; R = COMe) (7·0 g., 1 mol.) in dry benzene (50 c.c.) containing ethanol (0·25 c.c.) was added to powdered sodium (0·50 g., 1·05 mols.) under benzene (50 c.c.). The solution became brown, the temperature rose, and hydrogen was evolved. After ½ hour, the mixture was refluxed for 4 hours, by which time all the sodium had reacted. The bulk of the solvent was removed on the steam-bath at reduced pressure, the dark-brown residue dissolved in cold water (100 c.c.), and non-acidic material removed with ether and discarded. The aqueous layer was acidified with concentrated hydrochloric acid and the precipitated oil taken up in chloroform. The extract was washed with cold aqueous sodium

hydrogen carbonate until no further colour was removed; the organic layer was then evaporated to give the *keto*-ester as a light-brown oil (2·1 g., 34·7%) which gave a gummy dinitrophenylhydrazone and an intense purple colour with aqueous ferric chloride. Decomposition accompanied bulk distillation; a small specimen distilled for analysis had b. p. 120—130° (air-bath temp.)/0·1 mm., and was a clear very viscous syrup (Found: C, 53·9; H, 6·7. $C_{13}H_{19}O_6N$ requires C, 54·7; H, 6·7%).

Triethyl N-*Benzoyl*-(N-*carboxymethyl*)*glutamate* (VIII; R = COPh).—When the crude ester (VIII; R = H) (17·9 g., 1 mol.) in dry pyridine (50 c.c.) was treated with benzoyl chloride (9·6 g., 1·1 mols.), the temperature of the mixture rose rapidly, and after ½ hour on a steam-bath, the red solution was poured into water (250 c.c.), acidified with hydrochloric acid, and the non-basic oil extracted with ether. Distillation of the dried extract (Na_2SO_4) gave the *benzoate* as a viscous straw-coloured oil (17·8 g., 74%), b. p. 195°/0·15 mm. (Found: N, 3·9. $C_{20}H_{27}O_7N$ requires N, 3·6%). When the distilled material (VIII; R = H) was used, the desired product was accompanied by a lower-boiling substance, b. p. *ca.* 150—160°/0·2 mm.

Diethyl 1-*Benzoyl*-5-*ketopiperidine*-2 : 4-*dicarboxylate* (IX; R = COPh).—The triester (VIII; R = COPh) (17·8 g., 1 mol.) containing ethanol (0·25 c.c.) was added to powdered sodium (1·2 g., 1·05 mols.) under benzene (50 c.c.). Reaction took place at the b. p., with evolution of hydrogen, and after 5 hours' refluxing the sodium had disappeared. The solvent was then removed at low pressure and the product isolated as in the case of the corresponding acetyl derivative. The *keto*-ester, an orange-coloured oil (7·8 g., 49·6%), with an intense purple ferric chloride colour, gave a gummy dinitrophenylhydrazone. Distillation on a large scale was accompanied by much decomposition, but a small quantity had b. p 190° (air-bath temp.)/0·1 mm. (Found: C, 62·6; H, 6·3; N, 4·2. $C_{18}H_{21}O_6N$ requires C, 62·3; H, 6·1; N, 4·1%). A common property of the cyclic esters in this series is the possession of a slight but extremely offensive and persistent smell only apparent in high dilution, and with an affinity for wool and leather.

5-*Ketopiperidine*-2-*carboxylic Acid* (X).—The above ester (2·7 g.) was refluxed with hydrochloric acid (25 c.c., 15%) for 6 hours, by which time all but a small amount of dark oil had passed into solution; during the initial ½ hour of heating, evolution of carbon dioxide was observed. On cooling, benzoic acid separated and this together with other insoluble impurities was removed by shaking with ether. The aqueous residue was evaporated to dryness on a steam-bath under reduced pressure, taken up in methanol (5 c.c.), and acetone added until the solution became cloudy. On being kept overnight at 0°, the keto-acid *hydrochloride* separated as rosettes of stout needles, m. p. 142° (decomp.) (yield 1·2 g., theory 1·43 g.). The quantity of material isolated suggested that solvent was present in appreciable quantity, and this was confirmed by analysis, which indicated the presence of both acetone and methanol of crystallisation, and by the hydrogen uptake of the acetone on quantitative hydrogenation (Found: C, 44·2, 44·9; H, 7·5, 7·4. $C_6H_9O_3N,HCl,CH_4O,C_3H_6O$ requires C, 44·5; H, 7·6%). Removal of the solvent by heat led to decomposition of the material.

The *dinitrophenylhydrazone hydrochloride* was prepared by boiling the crude keto-acid hydrochloride (which had not been treated with acetone) with 2 : 4-dinitrophenylhydrazine in ethanol. The derivative separated as a microcrystalline powder, very sparingly soluble in alcohol, and was purified by dissolution in a large volume of methanol from which it separated on evaporation to a small bulk as an orange powder, m. p. 193° (decomp.) (Found: C, 40·5; H, 4·1. $C_{12}H_{13}O_6N_5,HCl$ requires C, 40·1; H, 3·9%).

5-*Hydroxypiperidine*-2-*carboxylic Acid* (XI).—The recrystallised ketone hydrochloride (1 g.), dissolved in methanol (50 c.c.), was shaken with hydrogen at room temperature and pressure over Adams's platinum catalyst (0·1 g.). Uptake of hydrogen was rapid at first but was not complete in under 3 hours (absorption 165 c.c., calc. 166·3 c.c.). After removal of the catalyst, evaporation of the solvent gave the *hydroxy-hydrochloride* as a dry crystalline solid (0·62 g., 92%) (in a subsequent experiment in which hydrogenation was slower the product at this stage was of waxy consistency), very slowly separating from methanol–acetone in microscopic leaflets, m. p. 240° (decomp.) (Found: C, 40·2; H, 6·7; N, 7·2. $C_6H_{11}O_3N,HCl$ requires C, 39·7; H, 6·7; N, 7·7%).

Attempts to dehydrate this material to an unsaturated compound by various methods, *e.g.*, treatment with thionyl chloride in pyridine or dimethylaniline, heating it with potassium hydrogen sulphate, and warming it with syrupy phosphoric acid, were unsuccessful. Similar experiments on the 1-benzoyl methyl ester were also unavailing.

Diethyl 1-*Benzoyl*-5-*hydroxypiperidine*-2 : 4-*dicarboxylate* (XII).—The keto-ester (IX; R = COPh) (12·4 g.) in ethanol (150 c.c.) was hydrogenated at 120—130°/140 atms. for 2 hours, Raney nickel (2 g.) catalyst being used. After removal of the catalyst and solvent, the alcohol (XII) (11 g.), which was free from ketone (absence of ferric chloride colour), was a very pale-yellow hygroscopic oil. On a small scale it could be distilled without appreciable decomposition at 200—210° (air-bath temp.)/0·1 mm., as a nearly colourless gum with a slight green fluorescence (Found: C, 62·4; H, 6·9. $C_{18}H_{23}O_6N$ requires C, 61·9; H, 6·6%). When cold the viscosity of this substance was such that it could only with difficulty be dented with a glass rod.

Diethyl L-1-*Benzoyl*-1 : 2 : 3 : 6-*tetrahydropyridine*-2 : 4-*dicarboxylate* (XIII).—The above alcohol (XII) (3 g.) was treated at −10° in pyridine or dimethylaniline (2 c.c.) with thionyl chloride (2 g.). The solution became very dark, and after 1 hour at 0° and then at 100°, the resultant thick tarry mass was treated with water (25 c.c.) and extracted with ether. The extract was washed with dilute hydrochloric acid, aqueous sodium carbonate, and finally water. The red ether solution so obtained was dried (Na_2SO_4) and evaporated to give a dark-red viscous oil (2·5 g., 87·8%), freely soluble in ether in contrast to (XII) which was only very sparingly soluble in this solvent. Distillation, which was accompanied by decomposition, gave an orange-coloured hygroscopic, very viscous oil, b. p. 190° (air-bath temp.)/0·1 mm. (Found: N, 4·6. $C_{18}H_{21}O_5N$ requires N, 4·2%).

Attempted dehydration of (XII) by heating it with potassium hydrogen sulphate was unsuccessful, unchanged substance being recovered.

L-1 : 2 : 3 : 6-*Tetrahydropyridine*-2 : 4-*dicarboxylic Acid* (XIV).—(i) The ester (XII) (2·1 g.) at 180—200° was treated with a stream of dry hydrogen chloride for 2 hours (cf. McElvain and Stork, *loc. cit.*). After cooling, the resinous product was hydrolysed with boiling hydrochloric acid (50 c.c.; 15%) for 3 hours. Non-basic material and benzoic acid were removed with ether, and on evaporating the aqueous solution a brown gum was obtained which slowly solidified at 0° under acetone. The material so obtained was difficult to purify, but the pure amino-acid hydrochloride identical with that from (ii) below was obtained in small yield (0·35 g., 27%) by recrystallisation from methanol–acetone.

(ii) The unsaturated diester (XIII) (2·0 g.) was refluxed with hydrochloric acid (50 c.c.; 15%) for 4 hours, by which time all but a small quantity of material had passed into solution. On cooling, benzoic acid separated and this together with other non-basic material was removed with ether. Evaporation of the aqueous layer gave a light-brown gum which solidified on trituration with acetone giving the amino-acid *hydrochloride* as a crystalline solid (0·9 g., 72%). This was recrystallised only with difficulty and with poor recovery from methanol–acetone in nearly colourless rosettes of small prisms, m. p. 241° (decomp.) (Found : C, 40·9; H, 5·1; N, 6·5. C_7H_9N,HCl requires C, 40·4; H, 4·8; N, 6·75%).

The *tetrahydropyridinedicarboxylic* acid was obtained by treatment of the hydrochloride in aqueous solution with a small excess of silver carbonate. Evaporation of the aqueous layer from which silver chloride and excess of carbonate had been removed, gave a solid which crystallised well from a little water in long prisms, m. p. 256° (decomp.) (Found : N, 8·4. $C_7H_9O_4N$ requires N, 8·2%).

Attempted decarboxylation by boiling quinoline containing copper powder, or by dropping the acid into boiling diphenyl ether was unsuccessful.

L-1 : 2 : 3 : 6-*Tetrahydropyridine*-2-*carboxylic Acid* (Baikiain) (III).—(i) The hydrochloride of the tetrahydropyridinedicarboxylic acid (XIV) was heated in a sealed tube with concentrated hydrochloric acid at 180—190° for 3 hours. The brown solution was evaporated and the tarry residue heated at 250°/0·5 mm. A small sublimate was obtained which was largely ammonium chloride, but when subjected to partition chromatography on a paper strip, phenol saturated with water being used as the mobile phase, the sublimate gave on development with ninhydrin a spot identical in position and appearance with that shown by baikiain hydrochloride run as a standard on the same paper. The acid (XIV) gave a characteristic orange-coloured spot with ninhydrin, quite distinct from that given by baikiain.

(ii) Pyrolysis at 250°/0·5 mm. of the amino-acid (XIV) alone and mixed with copper bronze resulted in decomposition with the formation of a sublimate mainly of a tarry nature. However, careful resublimation revealed the presence of a minute amount of solid material in which the presence of baikiain was shown by partition chromatography.

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