35. *The 4-Hydroxypipecolic Acid from* Acacia *species, and its Stereoisomers.*

By J. W. Clark-Lewis and P. I. Mortimer.

4-Hydroxypipecolic acid has been isolated on a preparative scale from *Acacia oswaldii* leaves and separated from accompanying acids through the ether-soluble *N*-nitroso-acid. Hydrolysis of this derivative and separation on an ion-exchange column gave (−)-pipecolic acid and the hydroxy-acid, which was shown by unequivocal degradations to be (−)-*trans*-4-hydroxy-L-pipecolic acid. This acid has been converted by stereospecific transformations into *cis*-4-hydroxy-L- and -D-pipecolic acid, so that three of the four optically active forms of 4-hydroxypipecolic acid are now available.

4-Hydroxypipecolic acid (I) was first isolated in 1955 by Virtanen and Kari [1] from some *Acacia* species and subsequent reports concern its structure [2,3,4] and isolation from other sources, *e.g.*, *Armeria* species,[2,3] heartwood of *Acacia excelsa*, and heartwood and sapwood of *A. mollissima*.[4] *Acacia oswaldii* leaves [4] offer a convenient source from which the acid has been isolated in 20—25 g. batches, and it is here free from the better known 5-hydroxypipecolic acid.[5,6] The leaves were extracted with 80% ethanol; the total nitrogenous acids (1·65%) were isolated on an ion-exchange column, and imino-acids were separated from amino-acids through the *N*-nitroso-derivatives as described for 5-hydroxypipecolic acid by Witkop and Foltz,[6] and again purified on an ion-exchange column. This gave (−)-pipecolic acid and a product, $C_6H_{11}O_3N$, melting at 294° (decomp.), 24° above the recorded [1] m. p. of 4-hydroxypipecolic acid. The latter product was characterised by various new derivatives; the ready cyclisation of the 1-phenylcarbamoyl compound to a hydantoin derivative revealed the α-imino-acid structure. This was confirmed by reduction of the natural acid with hydriodic acid and red phosphorus to pipecolic acid (II) (16%), the reduction being accompanied by racemisation as in the case of hydroxyproline.[7] When heated with aqueous barium hydroxide the natural acid was partially epimerised, and the new product was chromatographically indistinguishable from synthetic *cis*-4-hydroxypipecolic acid supplied by Dr. Vanderhaeghe. The synthetic acid was similarly epimerised to a mixture of *cis*- and *trans*-acids, and both these were distinguishable from 3- [8] and 5-hydroxypipecolic acid [6] by paper chromatography.[4] The position of the hydroxyl group in 4-hydroxypipecolic acid was then confirmed by decarboxylation of the acid in acetophenone by the method introduced by Chatelus.[9] This gave dimorphic 4-hydroxypiperidine (III) smoothly, but in lower yield (37%) than reported for decarboxylation of acyclic amino-acids,[9] and this completed the chemical proof of the 4-hydroxypipecolic acid structure (I).

The *trans*-configuration (IV) was indicated by the compound's differing chromatographically from *cis*-4-hydroxypipecolic acid prepared by hydrogenation of 4-hydroxypicolinic acid. Moreover the higher R_F value of the *trans*- than of the *cis*-acid agreed with the assumed conformations (2-*eq*,4-*ax* and 2-*eq*,4-*eq* respectively), and the acid and its derivatives showed no tendency to lactonise. (−)-*trans*-4-Hydroxypipecolic acid belongs to the L-series of imino-acids, as was expected from its occurrence with (−)-L-pipecolic acid and confirmed by its less negative rotation in aqueous hydrochloric acid than in water.

[1] Virtanen and Kari, *Acta Chem. Scand.*, 1955, **9**, 170.
[2] Fowden, *Biochem. J.*, 1958, **70**, 629.
[3] Virtanen and Gmelin, *Acta Chem. Scand.*, 1959, **13**, 1244.
[4] Clark-Lewis and Mortimer, *Nature*, 1959, **184**, 1234.
[5] Virtanen and Kari, *Acta Chem. Scand.*, 1954, **8**, 1290; Grobbelaar, Pollard, and Steward, *Nature*, 1955, **175**, 703.
[6] Witkop and Foltz, *J. Amer. Chem. Soc.*, 1957, **79**, 192.
[7] Fischer, *Ber.*, 1902, **35**, 2660.
[8] Plieninger and Leonhäuser, *Chem. Ber.*, 1959, **92**, 1579.
[9] Chatelus, *Compt. rend.*, 1959, **248**, 690.

The acid thus conforms to Lutz and Jirgensons's empirical rule[10,11] that protonation of the carboxyl group in the L-series causes a decrease in the negative rotation (rotation becomes more positive, see Table); the validity and scope of this rule have been examined,[11] and it has proved useful with 5-hydroxypipecolic acid.[6] A further regularity apparent from the

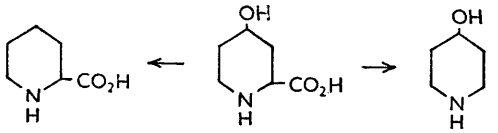
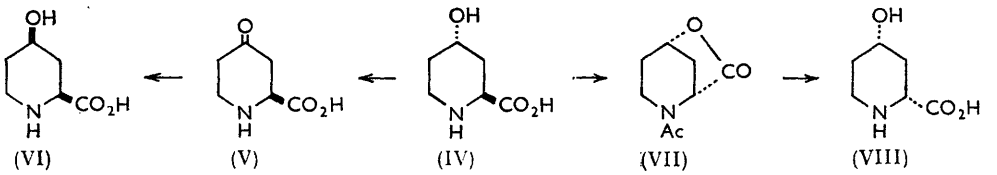


Table is that the change in molecular rotation is greater in the *cis*- than in the *trans*-series, which supports the assignment to the natural acid of the (—)-*trans*-4-hydroxy-L-pipecolic acid structure with the absolute configuration shown (IV).

Acids of L-series	$[M]_D$ in H_2O	$[M]_D$ in H_2O with 1 equiv. of mineral acid	$[M]_D$ in 5N-HCl	$[M]_D$ in acid minus $[M]_D$ in H_2O
Proline [10b]	−100°	−71°	—	+29°
Hydroxyproline [11]	−100	—	−66°	+34
cis-4-Hydroxyproline	−78	—	−25	+53
Pipecolic	−33	—	−14	+19
trans-4-Hydroxypipecolic	−19	—	+4	+23
cis-4-Hydroxypipecolic	−28	—	+11	+39
trans-5-Hydroxypipecolic [6]	−34	−20	—	+14
cis-5-Hydroxypipecolic [6]	−45	−17	—	+28

The *trans*-L-configuration (IV) was then substantiated by transformation of the (—)-4-hydroxypipecolic acid *via* the keto-L-acid (V) into (—)-*cis*-4-hydroxy-L-pipecolic acid (VI), and *via* the *N*-acetyl-lactone (VII) into (+)-*cis*-4-hydroxy-D-pipecolic acid (VIII). The

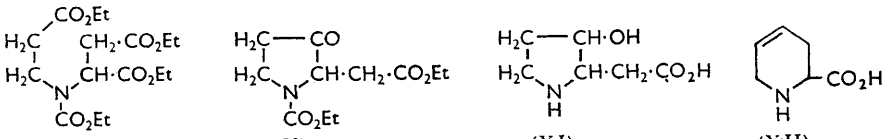


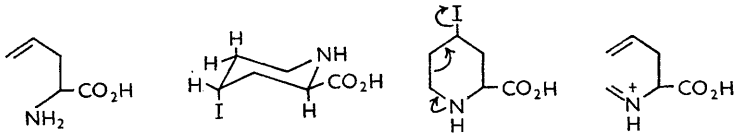
keto-acid (V) formed by oxidation of the 4(*ax*)-hydroxy-2(*eq*)-carboxylic acid (IV) was expected to retain the 2(*eq*)-conformation, and reduction of the unhindered carbonyl group by sodium borohydride then gave predominantly the expected[12] 4(*eq*)-hydroxy-2(*eq*)-carboxylic acid, *viz.* (—)-*cis*-4-hydroxy-L-pipecolic acid (VI), chromatographically identical with the synthetic *cis*-acid. Oxidation was effected with the unacylated imino-acid (IV) so that paper chromatography could be used as an aid in determining the conditions for both oxidation (with chromic acid) and isolation of products, and in this way the keto-acid (V) was obtained in 36% yield and β-alanine, the only other ninhydrin-positive oxidation product, was isolated as the *N*-phenylcarbamoyl derivative (2·2%). 4-Oxo-L-pipecolic acid (V) was found to be stable in acid solution and decomposed only slowly in neutral or in alkaline solution, in marked contrast to the behaviour of 4-oxoproline,[13] 5-oxopipecolic acid,[14] and *N*-benzyloxycarbonyl-5-oxopipecolic acid.[6] Moreover the stability of the acid precludes a β-keto-acid structure and thus provides independent confirmation of the location of the carboxyl group.

[10] Lutz and Jirgensons, *Ber.*, (*a*) 1930, **63**, 448, (*b*) 1931, **64**, 1221.
[11] Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1955, **77**, 716.
[12] Barton, *J.*, 1953, 1027; Dauben, Fonken, and Noyce, *J. Amer. Chem. Soc.*, 1956, **78**, 2579.
[13] Patchett and Witkop, *J. Amer. Chem. Soc.*, 1957, **79**, 185.
[14] King, King, and Warwick, *J.*, 1950, 3590.

The disposition of hydroxyl and carboxyl groups in (—)-*trans*-4-hydroxy-L-pipecolic acid (IV) is unfavourable for lactone formation, but the acid suffered inversion at the 2-position with formation of the *N*-acetyl-D-lactone (VII) when boiled with acetic anhydride in acetic acid. Hydrolysis of the lactone then gave (+)-*cis*-4-hydroxy-D-pipecolic acid (VIII), enantiomorphous with (—)-cis-L-acid (VI) obtained by reduction of the keto-acid with sodium borohydride. Racemisation at the α-carbon atom in *N*-acyl-α-amino-acids on treatment with acetic anhydride is well known and was used to epimerise hydroxyproline;[15] it is usually held to proceed through an oxazolone (see, *e.g.*, footnote 15, ref. 6), although this clearly is not so in the case of phthaloyl-L-glutamic anhydride which is similarly racemised.[16] Inversion at the 2-carbon atom is confirmed in the present case by application of Hudson's lactone rule:[17] $[M]_D$ of the lactone (VII) is +306° (1% in ethanol) and is more positive by 208° than $[M]_D$ +98° (1% in ethanol) of the *N*-benzoyl (in lieu of *N*-acetyl) derivative of the acid (VIII) (rotation measured with the enantiomorph, VI). A mixture of the *trans*-L- and the *cis*-D-acid was formed by partial inversion at the 2-position when the L-acid was heated with aqueous barium hydroxide or when *trans*-*N*-benzoyl-4-hydroxy-L-pipecolic acid was heated above its m. p. and then hydrolysed. Crystallisation of the copper salts gave a series of fractions each containing approximately equal amounts of *cis*- and *trans*-salt so that this method, which proved so effective with the epimeric hydroxyprolines,[18] could not be used to separate *cis*- and *trans*-4-hydroxypipecolic acid.

Synthesis of (±)-*cis*-4-hydroxypipecolic acid by catalytic hydrogenation of 4-hydroxypicolinic acid was achieved in very low yield by us (1·4% isolated as the hydrochloride) and by Fowden.[2] Yields were not improved by using newly prepared 4-benzyloxypicolinic acid because catalytic hydrogenation led first to 4-hydroxypicolinic acid through hydrogenolysis of the benzyl group. An alternative synthesis was therefore attempted by condensing *N*-ethoxycarbonyl-β-alanine ethyl ester with diethyl fumarate, giving the ester (IX), in the hope that Dieckmann cyclisation would yield a piperidone derivative. Cyclisation, however, gave a pyrrolidone which on hydrolysis, decarboxylation of the β-keto-acid, and re-esterification gave the keto-ester (X). Reduction of the keto-ester by sodium borohydride and hydrolysis then gave 3-hydroxypyrrolidine-2-acetic acid (XI).


(IX) (X) (XI) (XII)


(XIII) (XIV) (XV) (XVI)

Paper chromatography greatly aided this investigation and all structural features of *trans*-4-hydroxypipecolic acid (IV) except the absolute configuration were in fact known from application of this analytical technique to reactions effected on a few milligrams of material, before being confirmed by isolation of products or by alternative degradations. A particularly interesting, and at first puzzling, series of observations was made on the reduction of the acid with hydriodic acid. This gave pipecolic acid, as already noted, and

[15] Robinson and Greenstein, *J. Biol. Chem.*, 1952, **195**, 383.
[16] Jackson, Thesis, Nottingham, 1951.
[17] Witkop, *Experientia*, 1956, **12**, 372.
[18] (*a*) Leuchs and Felser, *Ber.*, 1908, **41**, 1726; (*b*) Wieland and Wintermeyer, *Chem. Ber.*, 1957, **90**, 1721.

two other ninhydrin-positive components regarded as iodo-derivatives (cf. hydroxyproline [18a]) which were also observed by Virtanen and Kari [1] and by Fowden.[2] Although unchanged in R_F after addition of ammonia, both disappeared when the reduction mixture was treated with silver carbonate and instead there appeared baikiain [14] (XII) and 2-aminopent-4-enoic acid (XIII). The former was chromatographically indistinguishable from natural baikiain, and the latter was recognised on hydrogenation of the silver-treated reduction mixture to 2-aminopentanoic acid (norvaline); the identification was then confirmed by paper-chromatographic comparison with synthetic material (XIII). Baikiain, pipecolic acid, α-aminopent-4-enoic acid, and norvaline give characteristically different colours with spray reagents, and this aided identification. It seems clear that the iodo-compounds, possessing different R_F values, are epimeric 4-iodopipecolic acids with iodine in 4(*ax*)- (XIV) and 4(*eq*)-orientation. *trans*-Elimination of hydrogen iodide from the former (XIV) accounts for the formation of baikiain, the preferential elimination of the 5- instead of the 3-hydrogen atom apparently being controlled by the carboxyl group (or ion). The iodine in the epimer (XV) is unfavourably placed for *trans*-1,2-elimination with hydrogen, but movement of the electrons of the 5,6-bond initiated by development of a 4-carbonium ion, and assisted by the nitrogen lone-pair electrons, would lead to the precursor (XVI) of 2-aminopent-4-enoic acid. A similar elimination with loss of a ring-carbon atom is entailed in the conversion of quinine into niquine,[19] and a close analogy is provided by the ring fission which occurs when 3β-chlorotropane (but not the 3α-isomer) is treated with potassium cyanide.[20]

Experimental

Paper chromatograms were run with butan-1-ol–acetic acid–water (4 : 1 : 5) [21] except where otherwise indicated. Distances from the origin are quoted instead of R_F values in cases where the solvent was allowed to drip from the paper.

Materials.—*Acacia mollissima* was supplied by the South Australian Woods and Forests Department, and *A. excelsa* (herbarium specimen numbers 55 and 890) was obtained by courtesy of the Chemical Research Laboratories, C.S.I.R.O. *A. oswaldii* leaves were collected locally in the River Murray valley.

Isolation of Imino-acids.—(a) *From leaves.* The extractive obtained from undried *Acacia oswaldii* leaves (5·5 kg.) with 80% ethanol was dissolved in water and transferred in two portions to a column of sulphonated polystyrene (Zeokarb 225; 970 g.) and the amino-acids (95 g.) were eluted with 3N-ammonia. The imino-acids were extracted into ether as the *N*-nitroso-derivatives, as described for 5-hydroxypipecolic acid; [6] 2N-hydrochloric acid was placed in the receiver during the continuous ether-extraction to avoid rapid decomposition of the *N*-nitroso-compounds during evaporation of ether from the extract; the ether extract also contained much oxalic acid. The acidic solution of *N*-nitroso-compounds was boiled and evaporated, and the imino-acids were recovered by absorption on ZeoKarb 225 and elution with 3N-ammonia. The imino-acids (46 g.) were dissolved in boiling water (58 c.c.), and the hot solution was diluted with boiling ethanol, which caused separation of a brown deposit and slower crystallisation of 4-hydroxpipecolic acid. A solution of the crude product (20·7 g.) in water (500 c.c.) containing acetic acid (2·5 c.c.) deposited most of the brown pigment when stored at 0°, and the filtrate was completely decolorised by filtration through charcoal (2 g.) mixed with kieselguhr. The solution was evaporated and crystallisation of the residue from aqueous ethanol gave *trans*-4-hydroxy-L-pipecolic acid (17·8 g.), m. p. 285—286° (decomp.). Concentration of mother-liquors from the first crop (20·7 g.) and purification of the residue (6·9 g.) in the same way gave a further 5·2 g. (total 23 g., 0·4%), m. p. 287° (decomp.).

L-Pipecolic acid was obtained as the hydrochloride by evaporation of hydrochloric acid with the mother-liquors from the isolation of 4-hydroxypipecolic acid, and from the aqueous solution

[19] Turner and Woodward, in Manske and Holmes, "The Alkaloids," Academic Press, New York, 1953, Vol. III, p. 21—22.
[20] Archer, Lewis, and Zenitz, *J. Amer. Chem. Soc.*, 1958, **80**, 958; Archer, Bell, Lewis, Schulenberg, and Unser, *ibid.*, p. 4677.
[21] Partridge, *Biochem. J.*, 1948, **42**, 238.

remaining after ether-extraction of the *N*-nitroso-compounds by evaporation and extraction of the residue with ethanol. Crystallisation from aqueous ethanol gave (−)-pipecolic acid hydrochloride (6·5 g. from 17·3 kg. of leaves), m. p. 256—258°, $[\alpha]_D^{18}$ −10·5° (8% in H_2O).

Separation of 4-hydroxypipecolic acid and pipecolic acid was also achieved by selective elution from ZeoKarb 225, and the former was eluted with 0·2—0·4N-hydrochloric acid, while the latter (and proline) required 0·4—0·8N-acid. The mother-liquors from the isolation of 4-hydroxypipecolic acid from 20 kg. of leaves were treated in this way, and the column was finally washed with 1·6N-hydrochloric acid before elution with aqueous ammonia gave a *compound* (1·66 g.) crystallising from aqueous ethanol in needles (1·5 g.), m. p. 231—234° (decomp.), $[\alpha]_D^{24}$ +15° (1% in H_2O), $[\alpha]_D^{26}$ +22° (1% in 5N-HCl) [Found: C, 46·7; H, 7·8; N, 13·1. $(C_4H_8O_2N)_2$ requires C, 47·05; H, 7·9; N, 13·7%]. When chromatographed for 30 hr. the compound moved 10 cm. compared with pipecolic acid (23·5 cm.), *trans*-4-hydroxypipecolic acid (15·8 cm.), and *cis*-4-hydroxypipecolic acid (12·9 cm.). It gave a reddish-grey colour (red fluorescence under ultraviolet light) with ninhydrin at 100° and a green colour with isatin at 100°. A solution in 6N-hydrochloric acid was boiled for 16 hr., but no other ninhydrin-positive component was then found in the solution.

(b) *From* Acacia *sp. heartwoods and sapwoods.* Milled heartwood of *A. excelsa* (2094 g.) was extracted with light petroleum (b. p. 60—80°) for 12 hr. (1·8 g. of yellow waxy extractive), ether for 34 hr. (7·4 g. of extractive), acetone for 7 hr. (34 g. of extractive) and ethanol for 8 hr. Concentration of the ethanolic extract left a viscous residue (73 g.) which was diluted with water and filtered from an amorphous brown deposit. The filtrate was extracted with ethyl acetate for three periods of 8 hr. and the residue (20·7 g.) remaining from evaporation of the aqueous phase was dissolved in water (10 c.c.) which was then diluted with ethanol (100 c.c.). Crystallisation gave a mixture (9·85 g.) of 4-hydroxypipecolic and pipecolic acid contaminated with amorphous brown impurity. The mother-liquors and the crude imino-acid fraction were separately boiled with 6N-hydrochloric acid for 24 hr. to precipitate polyphenolic material, and the imino-acids were collected on a cation-exchange resin and eluted with 0·5N-ammonia. Evaporation of the eluates and repeated crystallisation of the residue from aqueous ethanol gave *trans*-4-hydroxypipecolic acid in colourless prisms (*ca.* 4·0 g., 0·19%), m. p. 294° (decomp.). The concentrated mother-liquors (2 g.) in water (4 c.c.) were chromatographed on Whatman 3 MM paper (7 sheets, each 25 cm. wide). (−)-Pipecolic acid (0·35 g., 0·017%), m. p. 273—275° (decomp.), $[\alpha]_D^{23}$ −25° (2% in H_2O), was extracted with boiling ethanol from appropriately dissected areas of the papers. 4-Hydroxypipecolic acid, similarly extracted from the chromatograms, failed to crystallise.

Similar extractions of other samples of *A. excelsa* heartwood gave crystalline 4-hydroxypipecolic acid (0·017—0·08%) and pipecolic acid (0·001—0·01%). 4-Hydroxypipecolic acid (0·01—0·03%) was also obtained from *A. mollissima* heartwood and sapwood.

(−)-trans-4-*Hydroxy*-L-*pipecolic Acid.*—The acid isolated as described above was obtained in clusters of prisms, m. p. 294° (decomp.) [lit.,[1] 270° (decomp.)], by repeated crystallisation from aqueous ethanol (Found, on material dried at 90° *in vacuo* over P_2O_5 for 1½ hr.: C, 49·7; H, 7·7; N, 9·8; *C*-Me, 0·1; OMe, 0·4. Calc. for $C_6H_{11}O_3N$: C, 49·6; H, 7·6; N, 9·65%). Samples isolated from three different batches had $[\alpha]_D^{20}$ −13° ± 0·4° (1% in H_2O); the acid has $[\alpha]_D^{16}$ +2·7° (1% in 5N-HCl) and $[\alpha]_D^{25}$ −6·0° (1% in N-NaOH). Virtanen and Gmelin [3] record $[\alpha]_D^{21}$ −12·5° (in H_2O) +0·34° (in N-HCl), and −18·5° (in N-NaOH). The acid did not react with periodic acid at a significant rate under the many conditions tested. The 1-(2,4-*dinitrophenyl*) *derivative* prepared by the general method [22] crystallised from aqueous ethanol in orange prisms, m. p. 183° (Found: C, 46·1; H, 4·2; N, 13·6. $C_{12}H_{13}O_7N_3$ requires C, 46·3; H, 4·2; N, 13·5%). 4-Hydroxypipecolic acid hydrochloride failed to crystallise. The *copper salt* crystallised from a concentrated aqueous solution (solubility *ca.* 1 in 2 at 100° and *ca.* 1 in 7 at room temperature) during 3 days in deep blue prisms, m. p. 229° (decomp.) (Found, on an air-dried sample: N, 6·5; CuO, 17·2; loss on drying at 90°, 16·2. $C_{12}H_{20}O_6N_2Cu,4H_2O$ requires N, 6·6; CuO, 18·7; H_2O, 17·0%). 4-Hydroxypipecolic acid on paper chromatograms sprayed with ninhydrin and heated at 100—110° for 5—10 min. gave a dull colour ranging from greyish-green to brownish-purple, with a dull red fluorescence in ultraviolet light, and with isatin under similar conditions it gave a green colour. The colours developed by the sprayed papers were found to vary markedly with the drying temperature. Thus at 80° isatin gave no colour, at 100° a faint purple, and at 115° a purple colour; these two colours became green during 24 hr. at room temperature. With

[22] Rao and Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 1328.

ninhydrin, heated at 80°, the initial yellow-brown colour became greyish-purple after 15 min. at room temperature.

(−)-trans-1-*Benzoyl*-4-*hydroxy*-L-*pipecolic Acid.*—The *benzoyl derivative* (60—70%) was prepared by Schotten–Baumann benzoylation at 0° and crystallised from aqueous ethanol in stout needles, m. p. 174°, $[\alpha]_D^{15}$ −54° (1% in EtOH) (Found: C, 62·8; H, 6·1; N, 5·6. $C_{13}H_{15}O_4N$ requires C, 62·6; H, 6·1; N, 5·6%). Recrystallisations from aqueous ethanol gave a poor recovery unless allowed to proceed at 0° for several days, and it was advantageous to remove as much ethanol as possible by boiling the solution after dilution. Benzoylation with an excess of benzoyl chloride did not yield the dibenzoate; heating the 1-benzoyl derivative above its m. p. caused epimerisation at the 2-carbon atom (see below).

(−)-trans-4-*Hydroxy*-1-*toluene*-p-*sulphonyl*-L-*pipecolic Acid.*—Toluene-*p*-sulphonyl chloride (0·95 g., 1·2 equiv.) in acetone (5 c.c.) was added to a solution of 4-hydroxypipecolic acid (0·58 g.) in water (5 c.c.) and 10N-sodium hydroxide (1·2 c.c.). The mixture was shaken for 15 min. before acidification with 10N-hydrochloric acid (1 c.c.), and was then extracted with ethyl acetate. The dried (Na_2SO_4) extract was concentrated, and re-evaporated after addition of benzene. Crystallisation gave (−)-trans-4-*hydroxy*-1-*toluene*-p-*sulphonyl*-L-*pipecolic acid* (0·7 g., 58%), m. p. 153—154° raised to m. p. 162° by recrystallisation (prisms) from ethyl acetate–benzene, $[\alpha]_D^{19}$ −16° (1% in EtOH) (Found: C, 51·8; H, 5·5; N, 4·5; S, 10·7. $C_{13}H_{17}O_5NS$ requires C, 52·1; H, 5·7; N, 4·7; S, 10·7%).

(−)-trans-4-*Hydroxy*-1-*phenylcarbamoyl*-L-*pipecolic Acid and* (−)-trans-4′-*Hydroxy*-3-*phenylpiperidino*(1′,2′:1,5)-*hydantoin.*—Phenyl isocyanate (0·6 g., 1·25 equiv.) was added in portions with shaking during 10 min. to a cold solution of 4-hydroxypipecolic acid (0·58 g.) in N-sodium hydroxide (4 c.c.). Diphenylurea was precipitated after the last addition, and acidification gave an oil which solidified (0·72 g., 68%). Reprecipitation by acidification of a cold solution in aqueous 2—3% sodium carbonate gave the *phenylurea* (0·48 g.), m. p. 181—197° after sintering at 176°, $[\alpha]_D^{26}$ −24·5° (1% in EtOH) unchanged after 24 hr. (Found: C, 59·0; H, 6·2; N, 10·5. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1; N, 10·6%). The phenylurea (1·49 g.) was dissolved in boiling water, and the filtered solution was concentrated (to 53 g.). Crystallisation gave the *piperidinohydantoin* in prisms (1·05 g.), m. p. 204—205°, $[\alpha]_D^{23}$ −53° (1% in EtOH) (Found: C, 63·5; H, 5·6; N, 11·5. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%). The hydantoin showed hydroxyl absorption at 2·88 μ and carbonyl absorption at 5·63 and 5·85 μ (Nujol; calcium fluoride prism). The hydantoin (0·61 g.) dissolved slowly (*ca.* 1 hr.) in N-sodium hydroxide (4·63 c.c.), and the solution after dilution to 10 c.c. then had $[\alpha]_D$ −17°, $[\alpha]_D$ −40° after 3 hr., and $[\alpha]_D$ −45·4° (6·1% in 2 equiv. of aqueous sodium hydroxide) after 24 hr. and after 48 hr. A solution of the hydantoin (0·1 g.) in N-sodium hydroxide (10 c.c.) was both prepared and kept at 25°, and optical rotations of the solution were observed at intervals measured from the first contact with alkali: $[\alpha]_D^{25}$ −17° after 1 hr., −30° (1½ hr.), −32·5° (2 hr.), −42° (3½ hr.), −43° (6½ hr.), and −43·5° (23 hr.). The acidified solution slowly deposited the hydantoin (0·047 g.), m. p. and mixed m. p. 205—206°.

(−)-trans-3-*Phenyl*-4′-*phenylthiocarbamoyloxypiperidino*(1′,2′:1,5)-2-*thiohydantoin.*—The derivative was prepared by Edman's method [23] but at room temperature: [24] 4-Hydroxypipecolic acid (0·725 g.) in 50% aqueous pyridine (25 c.c.) was adjusted to pH 10·0 with N-sodium hydroxide (1·4 c.c.), phenyl isothiocyanate (1·2 c.c.) was added, and the mixture was shaken vigorously. Aqueous sodium hydroxide was added as required (during the first 15 min.) to maintain pH 9·0. The alkaline solution was extracted with benzene, and the aqueous layer filtered through kieselguhr before acidification with 1·25N-hydrochloric acid. The granular yellow precipitate was collected, washed with water, suspended in 2N-hydrochloric acid (15 c.c.), and boiled for 2 hr. before evaporation to dryness under reduced pressure. Crystallisation from aqueous acetic acid gave the *thiohydantoin* (0·56 g., 28%), m. p. 201—202° raised by recrystallisation from ethanol (yellow plates) (0·45 g.) to 213—214° and then 214°, $[\alpha]_D^{22}$ −74° (0·2% in EtOH) (Found: C, 60·5; H, 4·9; N, 9·6; S, 15·6. $C_{20}H_{19}O_2N_3S_2$ requires C, 60·4; H, 4·8; N, 10·6; S, 16·1%).

Reduction of 4-*Hydroxypipecolic Acid by Hydriodic Acid.*—(a) *Isolation of pipecolic acid.* 4-Hydroxypipecolic acid (0·051 g.), red phosphorus (0·023 g.), and hydriodic acid (*d* 1·94; 1 c.c.) were heated in a sealed tube at 145° for 6 hr. and the colourless solution was evaporated under reduced pressure. Pipecolic acid (0·0076 g., 16%) was detected and estimated by

[23] Edman, *Acta Chem. Scand.*, 1950, **4**, 277.
[24] Ingram, *J.*, 1953, 3717.

quantitative paper chromatography (R_F 0·37). 4-Hydroxypipecolic acid (2 g.), red phosphorus (0·32 g.), and hydriodic acid (*d* 1·94; 20 c.c.) were heated in four sealed tubes at 150° for 12 hr. The solutions were combined and found to contain pipecolic acid (R_F 0·37) and components with R_F 0·48 and 0·56 (run for 12 hr.; solvent front moved 24 cm.). The solution was evaporated to remove hydriodic acid and the passed through a ZeoKarb 225 column (50 g.). Elution with ammonia gave pipecolic acid and several components of lower R_F; imino-acids were separated through their *N*-nitroso-derivatives which, after hydrolysis and passage through a ZeoKarb 225 column, gave imino-acids (0·77 g.) consisting of pipecolic acid and traces of other ninhydrin-positive substances. The mixture failed to crystallise and was therefore transferred to a ZeoKarb 225 column and eluted successively with 0·5N-hydrochloric acid (500 c.c.), N-hydrochloric acid (250 c.c.), water (500 c.c.), and 2N-ammonia (500 c.c.). The eluted fractions (*ca.* 70 c.c. each) were evaporated and examined by paper chromatography. Pipecolic acid was present in the later 0·5N-hydrochloric acid eluates, those with N-acid, and the first ammonia eluates. Fractions containing other ninhydrin-positive substances in addition to pipecolic acid were returned to the column and eluted with acid of more gradually increased strength. Combined pipecolic acid fractions were evaporated to dryness under reduced pressure, and recrystallisation of the residues from ethanol gave pipecolic acid hydrochloride (0·22 g., 8·5%), m. p. and mixed m. p. 256—258°, $[\alpha]_D$ −0·3° (6% in H_2O).

(b) *Formation of baikiain and 2-aminopent-4-enoic acid.* 4-Hydroxypipecolic acid (0·02 g.), red phosphorus (0·007 g.), and hydriodic acid (*d* 1·94; 0·2 c.c.) were heated at 145° for 12 hr. and the colourless solution was evaporated to dryness under reduced pressure. The residue was dissolved in water (2 c.c.) and examined by paper chromatography (16 hr.; solvent front moved 37 cm.). 4-Hydroxypipecolic acid (R_F 0·20) was absent and the chromatogram showed pipecolic acid (R_F 0·36) and components with R_F 0·52 and 0·58, apparently 4-iodopipecolic acids, which gave purple colours with ninhydrin and a blue-green and a faint pink colour respectively with isatin; the iodo-acids gave brown colours with cold neutral silver nitrate solution. In a second experiment the reduction mixture (from 0·02 g. of acid) was treated with an excess of freshly prepared silver carbonate and solids were removed by centrifugation. The aqueous phase was chromatographed (15 hr.; solvent front moved 39 cm.) and showed none of the component of R_F 0·52, a trace of the component of R_F 0·58, and two new components, 2-aminopent-4-enoic acid (R_F 0·37) and baikiain (R_F 0·31). The former acid gave a purple colour with ninhydrin at 110—115° (but yellow green [25] at 80—90°) and a purple colour fading to pink with isatin, and baikiain gave a grey-green colour with ninhydrin and pink with isatin. Pipecolic acid was obscured by the aminopentenoic acid. The identity of the two components (R_F 0·31 and 0·37) was confirmed by their being inseparable on paper chromatograms (44 hr.) from baikiain (29·4 cm.) and authentic 2-aminopent-4-enoic acid (33·8 cm. from origin); pipecolic acid moved 34·2 cm. The same solutions were chromatographed with butan-1-ol–benzyl alcohol (1 : 1) on paper buffered with M/15-phosphate buffer [26] (pH 7·5) for 40 hr. and the amino-acids were detected with ninhydrin and isatin sprays containing much acetic acid, followed by heating at 110°. The acids gave the following distinctive colours (distances from origin in brackets): pipecolic acid (13 cm.) bright blue-violet with ninhydrin, green with isatin; baikiain (10·7 cm.) bright yellow with ninhydrin, brick red with isatin; 2-aminopent-4-enoic acid (7·7 cm.) brown with ninhydrin, white with isatin; proline (6·0 cm.) orange with ninhydrin, blue with isatin; 4-hydroxypipecolic acid (3·7 cm.) grey-brown with ninhydrin, green with isatin; and 5-hydroxypipecolic acid (3·5 cm.) yellow (with grey edges) with ninhydrin, green with isatin.

(c) *Formation of "norvaline" by hydrogenation of the reduction products.* 4-Hydroxypipecolic acid (0·02 g.) was heated with red phosphorus (0·0035 g.) and hydriodic acid (*d* 1·94; 0·2 c.c.) at 145° for 9 hr., the solution was then evaporated on a steam-bath, and the residue was dried in a desiccator over sodium hydroxide. A solution of the residue in water (1 c.c.) was treated with freshly prepared silver carbonate until no further reaction occurred, and the insoluble silver salts were separated at the centrifuge. Half the supernatant solution was hydrogenated over Adams catalyst at room temperature and pressure for 3 hr. Chromatograms of the hydrogenated solution showed 2-aminopentanoic acid (norvaline), R_F 0·49 (purple with ninhydrin, not fluorescing in ultraviolet light; pink with isatin); pipecolic acid, R_F 0·33 (blue-purple with ninhydrin, bright red fluorescence under ultraviolet light; green with isatin);

[25] Schlögl and Fabitschowitz, *Monatsh.*, 1954, **85**, 1060.
[26] McFarren, *Analyt. Chem.*, 1951, **23**, 168; Burroughs, *J. Sci. Food Agric.*, 1957, **8**, 122.

4-hydroxypipecolic acid, R_F 0·16; and a minor component, R_F 0·10 (purple with ninhydrin, no fluorescence under ultraviolet light; pink with isatin). 4-Hydroxypipecolic acid (R_F 0·16), pipecolic acid (R_F 0·33), and baikiain (R_F 0·28) were used as standards on the same chromatogram. The norvaline spot at R_F 0·49 was very strong, and the colour reactions indicated the α-amino-acid structure; on paper chromatograms (15 hr.) it had R_F 0·49 and was distinguished from α-aminobutyric acid (R_F 0·28), leucine (R_F 0·54), and isoleucine (R_F 0·54). A mixture of the degradation product and authentic 2-aminopentanoic acid moved as a single component (R_F 0·45) on chromatograms run for 20 hr., but a mixture with valine was separated into 2-aminopentanoic acid (R_F 0·45) and valine (R_F 0·39). A mixture of valine (6·7 to 10·1 cm.) and norvaline (6·0 to 9·2 cm. from the origin) was incompletely separated when run on paper buffered with 0·067M-phosphate buffer at pH 7·5 developed with butan-1-ol–benzyl alcohol (1 : 1) for 30 hr.

Decarboxylation of 4-Hydroxpipecolic Acid.—Powdered 4-hydroxypipecolic acid (2 g.) in freshly distilled acetophenone (8 c.c.) was stirred and heated at 150° (bath) under nitrogen for 1½ hr. There was no apparent reaction and the temperature was raised to 190° for 1½ hr. The cooled mixture was diluted with ether (50 c.c.) and extracted with 2N-hydrochloric acid (10 c.c. and 2 × 5 c.c.). The acid extracts were washed with ether (20 c.c.), then evaporated under reduced pressure to a viscous residue (2·5 g.), to which were added water (2 c.c.) and potassium hydroxide (2 g.). Extraction with ether, evaporation, and distillation of the residue gave a colourless oil (0·52 g., 37%), b. p. *ca.* 219° (bath)/772 mm., which crystallised almost completely. The 4-hydroxypiperidine melted at 55—65° (capillary) and under the Leitz hot-stage microscope was seen to suffer a phase change at 57—60° to needles, m. p. 85—87°. Synthetic [27] 4-hydroxypiperidine similarly suffered a phase change at 59—60° and then melted at 85° alone or mixed with that obtained by decarboxylation of the imino-acid. The infrared spectra (2—11·5 μ; in $CHCl_3$) of the two samples were identical. The dimorphic 1-toluene-*p*-sulphonyl derivative crystallised from aqueous ethanol in needles, m. p. 114—115°, not depressed by admixture with synthetic 4-hydroxy-1-toluene-*p*-sulphonylpiperidine, m. p. 115—116°. The latter sometimes resolidified and melted again at 125°, and sometimes crystallised from aqueous ethanol in needles, m. p. 123—124° after sintering at 115° (capillary). Under the hot-stage microscope the substance had m. p. 125—126° with change of crystalline form at 117° (lit.,[28] m. p. 131—132°, from benzene).

Oxidation of 4-Hydroxy- to 4-Oxo-L-pipecolic Acid (V).—The following procedure was adopted after numerous trials to determine the optimum conditions for oxidation and isolation: 8N-Chromic acid in aqueous sulphuric acid [29] (7·5 c.c.) was added to 4-hydroxypipecolic acid (2·18 g., containing *ca.* 3% of pipecolic acid) in acetic acid (150 c.c.; purified over chromic acid), concentrated sulphuric acid (1·73 c.c.), and water (5 c.c.), and the temperature was kept below 20° by intermittent cooling. After 1½ hr. methanol (5 c.c.) was added and next day the solution was decanted from chromium salts. The combined solutions from four such oxidations were evaporated under reduced pressure to *ca.* 40 c.c. and, after dilution with water, sulphate was removed with barium carbonate, and excess of barium was removed with sulphuric acid. The solution was added to a ZeoKarb 225 column (200 g.; 2·5 × 53 cm.) (H^+ form) and eluted in the following fractions: 1, feed solution; 2, water (250 c.c.); 3—10, 0·1N-HCl (2 l.); 11—12, 0·2N-HCl (500 c.c.); 13—16, 0·4N-HCl (1 l.); 17—20, 0·8N-HCl (1 l.); 21—22, water (500 c.c.); 23—27, 3N-ammonia (1 l.). Fractions were evaporated on a steam-bath, and the residues were examined by paper chromatography (12 hr.; solvent front moved 27·4 cm.). Chromium was present in fractions 1—6 and 17—20; the keto-acid was present in fractions 11—16 and gave an elongated spot R_F 0·20; β-alanine (R_F 0·24) in fractions 16—20; and pipecolic acid (R_F 0·35) in fractions 19—20. The first ammoniacal eluate (fraction 24) contained some of each amino-acid, especially pipecolic acid. The mixtures of amino-acids in some of the fractions were separated on a 50 g. column of ZeoKarb 225. The keto-acid fractions were combined and evaporated to a thin syrup which on cooling gave *4-oxo-L-pipecolic acid hydrochloride monohydrate* (1·28 g.) in needles, decomp. 203°, $[\alpha]_D^{21}$ +3·8° (2% in H_2O) (Found, on an air-dried sample: loss at 80°, 8. $C_6H_9O_3N,HCl,H_2O$ requires H_2O, 9·1. Found, on a sample dried for 6 hr. *in vacuo*: C, 39·9; H, 5·65; N, 7·3; Cl, 19·4. $C_6H_9O_3N,HCl$ requires C, 40·1; H, 5·6; N, 7·8; Cl, 19·7%). Crystallisation after dilution of the mother-liquors with propan-2-ol gave a

[27] Bowden and Green, *J.*, 1952, 1164.
[28] Arndt and Kalischek, *Ber.*, 1930, **63**, 587.
[29] Djerassi, Engle, and Bowers, *J. Org. Chem.*, 1956, **21**, 1547 (footnote 10).

further 2·35 g. in needles, and further manipulation gave more keto-acid (total 4·3 g., 36%) which includes 4% recovered from the chromium salts. The free acid was obtained from the hydrochloride (0·4 g.) by elution from a ZeoKarb 225 column (28 g.) with N-ammonia. Evaporation under reduced pressure and dilution with propan-2-ol gave (−)-4-*oxo*-L-*pipecolic acid monohydrate* as prisms (0·19 g.), decomp. 240°, $[\alpha]_D^{23}$ −14·8° (1% in H_2O) (Found: C, 44·3; H, 7·0; N, 8·5. $C_6H_9O_3N,H_2O$ requires C, 44·7; H, 6·9; N, 8·7%).

β-Alanine fractions were collected and evaporation left a crystalline residue (0·59 g.) containing pipecolic acid which was converted by phenyl isocyanate into the phenylcarbamoyl derivatives (0·27 g.), m. p. 146—157°, from which pipecolic acid phenylhydantoin and *N*-phenylcarbamoyl-β-alanine (0·17 g., 1·5% from 4-hydroxypipecolic acid), m. p. 164—169°, were separated by dissolution in aqueous sodium carbonate. Authentic *N*-phenylcarbamoyl-β-alanine crystallised from water in blades, m. p. 173—174° (lit.,[30] m. p. 171°), mixed m. p. with the oxidation product 170—172°. Further *N*-phenylcarbamoyl-β-alanine (0·11 g., total 0·28 g., 2·2%) was recovered from the chromium salts.

4′-Oxo-3-phenylpiperidino(1′,2′:1,5)hydantoin.—Phenyl isocyanate (0·30 g., 1·25 equiv.) was added with shaking during 15 min. to an ice-cooled solution of 4-oxo-L-pipecolic acid hydrochloride (0·4 g.) in 0·5N-sodium hydroxide (8 c.c.). The filtrate from diphenylurea was acidified and next day the (−)-*hydantoin* was collected as needles, m. p. 187°, insoluble in aqueous sodium carbonate (Found: C, 63·8; H, 5·05; N, 11·3. $C_{13}H_{12}O_3N_2$ requires C, 63·9; H, 4·95; N, 11·5%). A solution in ethanol (0·1 g. in 25 c.c.), prepared by warming, showed mutarotation from −0·94° to −0·437° after 23 hr. The solution was boiled for 6 hr. and then concentrated to *ca.* 1 c.c. before slow crystallisation gave the recovered hydantoin (0·05 g.), m. p. and mixed m. p. 187°. The recovered material had $[\alpha]_D^{23}$ −55° (0·16% in EtOH, dissolved with heating). Recrystallisation of the phenylhydantoin (0·15 g.) from ethanol (10 c.c.) as rapidly as possible gave needles (0·102 g.), m. p. 187°. A solution in ethanol prepared in the cold exhibited $[\alpha]_D^{23}$ −87° (0·366% in EtOH) 10 min. after preparation.

(−)-cis-4-*Hydroxy*-L-*pipecolic Acid* (VI).—A solution of 4-oxo-L-pipecolic acid hydrochloride monohydrate (2 g.) in water (20 c.c.) was adjusted to pH 9·0 with N-potassium hydroxide (16·6 c.c.) and cooled to 20° while an aqueous solution of sodium borohydride (0·112 g., 1·2 equiv.) was added in portions. The temperature of the solution rose after each adition, and the solution was kept at room temperature for 1 hr. and then just acidified with acetic acid. The solution was added to a ZeoKarb 225 column (28 g., 1·2 × 27 cm.) (H^+ form), and the amino-acids were recovered with N-ammonia. Evaporation gave a thin syrup which crystallised [1·05 g.; m. p. 265° (decomp.)] after addition of ethanol. A second crop (0·23 g.) had m. p. 250—263° (decomp.) and left 0·27 g. in the mother-liquors. Paper chromatography (40 hr.) showed that the crystalline fractions contained mainly *cis*-acid with a trace of the natural isomer, and the latter predominated in the mother-liquor. (−)-cis-4-*Hydroxy*-L-*pipecolic acid monohydrate* (78%) crystallised from aqueous ethanol in plates, m. p. 265° (decomp.), $[\alpha]_D^{23}$ −17° (1·1% in H_2O), $[\alpha]_D$ +7° (1·1% in 5N-HCl) (Found: C, 43·6; H, 8·1; N, 8·5; loss on drying at 80° *in vacuo*, 11·6. $C_6H_{11}O_3N,H_2O$ requires C, 44·2; H, 8·0; N, 8·6; H_2O, 11·0%). The acid sometimes crystallised from aqueous ethanol as a *dihydrate*, m. p. 265° (decomp.) (Found: C, 39·7; H, 8·2; N, 7·7; loss on drying at 80° *in vacuo*, 19·0. $C_6H_{11}O_3N,2H_2O$ requires C, 39·8; H, 8·3; N, 7·7; loss, 19·9%). The *copper salt* crystallised from water in deep blue plates, m. p. 245° (decomp.), which did not lose weight over anhydrous calcium chloride (Found: N, 6·6; CuO, 16·9. $C_{21}H_{20}O_6N_2Cu,4H_2O$ requires N, 6·6; CuO, 18·7%). The N-(2,4-*dinitrophenyl*) *derivative* (62%) crystallised from aqueous ethanol in yellow prisms, m. p. 134° (Found: C, 45·0; H, 4·2; N, 13·1. $C_{12}H_{13}O_7N_3,\frac{1}{2}H_2O$ requires C, 45·0; H, 4·4; N, 13·1%).

(−)-cis-1-*Benzoyl*-4-*hydroxy*-L-*pipecolic Acid.*—Benzoyl chloride (0·15 g., 1·1 equiv.) was added in portions to the *cis*-acid monohydrate (0·163 g.) in ice-cooled 0·7N-sodium hydroxide (3·2 c.c.), with shaking, the filtrate was acidified, and light petroleum added to dissolve benzoic acid. After 14 hr. at 0° the N-*benzoyl derivative* crystallised from the aqueous layer in blades (0·119 g.), m. p. 104°, $[\alpha]_D^{23}$ −39·5° (1% in EtOH) (Found: C, 58·5; H, 6·5; N, 5·2. $C_{13}H_{15}O_4N,H_2O$ requires C, 58·4; H, 6·4; N, 5·2%). The same product was obtained when 2·2 equiv. of benzoyl chloride were used. The ethanolic solution (1%; 10 c.c.) used in the polarimeter was evaporated after addition of water, and the derivative crystallised slowly at 0° in a *form* (blades) (0·076 g., 76% recovery), m. p. 191°, $[\alpha]_D^{26}$ −38·5° (1% in EtOH) (Found: C, 58·7; H, 6·4; N, 5·2%). It is thus dimorphic and a mixture of the first form (m. p. 104°) with

[30] Fischer and Leuchs, *Ber.*, 1902, **35**, 3787.

the second (m. p. 191°) melted at 191°. Both forms dissolved readily in aqueous sodium hydrogen carbonate and gave similar infrared spectra (in $CHCl_3$; calcium fluoride prism) in the recorded range 2—6·5 μ.

4-Benzyloxypicolinic Acid.—Methyl 4-chloropicolinate (37%), m. p. 48—52°, was prepared according to Mosher and Look,[31] who report m. p. 57—58° and 71% yield. Sodium (1 g.) was dissolved in benzyl alcohol (30 c.c.) almost at the b. p. (to reduce its viscosity) and the cold solution was added in portions, with stirring, to a solution of methyl 4-chloropicolinate (3·43 g.) in benzyl alcohol. The mixture was boiled gently for 45 min. and was then cooled (it became gelatinous), and water (50 c.c.), ether (100 c.c.), and 2N-hydrochloric acid (50 c.c.) were added. The mixture was shaken and the ether layer was washed twice with 2N-hydrochloric acid and discarded. The acidic extracts were combined and washed with ether, and 5N-sodium hydroxide (50 c.c.) was added. This caused separation of crystals and the mixture was boiled to remove dissolved ether and then stored at 0°. Next day the crystalline sodium 4-benzyloxypicolinate (3·65 g.) was collected and dissolved in hot water (20 c.c.), and acetic acid (1·3 c.c., a slight excess) was added. The acid (2·95 g.) crystallised at 0° during 2 days and recrystallisation from ethanol (charcoal) gave *4-benzyloxypicolinic acid* (2·4 g., 52%) in prisms, m. p. 172° (Found: C, 68·4; H, 5·0; N, 5·9. $C_{13}H_{11}O_3N$ requires C, 69·1; H, 4·8; N, 6·1%). The *hydrochloride monohydrate* (83%) crystallised in needles, m. p. 158° raised to 162° by recrystallisation from 5N-hydrochloric acid (Found: C, 55·2; H, 5·0; N, 4·6; Cl, 13·2. $C_{13}H_{19}O_3N,HCl,H_2O$ requires C, 55·0; H, 5·0; N, 4·9; Cl, 13·5%). When heated above its m. p. crystals appeared in the melt (at *ca.* 205°) and melted again at 238—250° (decomp.). The hydrochloride (0·39 g.) was heated at 200°, whereupon it melted and resolidified, and gave a liquid distillate with the odour of benzyl chloride. The solid product was washed with light petroleum (b. p. 60—80°) before crystallisation from water gave 4-hydroxypicolinic acid (0·15 g., 73%) in prisms, m. p. 258° (decomp.) alone or mixed with that obtained by hydrogenolysis of 4-benzyloxypicolinic acid.

Hydrogenation of 4-Benzyloxypicolinic Acid.—Hydrogenation of 4-benzyloxypicolinic acid (1 g.) in 5N-hydrochloric acid (20 c.c.) at room temperature and pressure over Adams catalyst (0·1 g.) proceeded rapidly with complete dissolution of the compound in 2 hr. Hydrogen uptake ceased after 29 hr. and the solution contained pipecolic acid as the only ninhydrin-positive or isatin-positive material. The filtered and concentrated solution deposited 4-hydroxypicolinic acid (0·52 g., 85%) in prisms of m. p. varying between 255° and 258° (Found: C, 51·7; H, 3·6; N, 10·1. Calc. for $C_6H_5O_3N$: C, 51·8; H, 3·6; N, 10·1%). Hydrogenation was inhibited in 1·5N-ammonia but in acetic acid at 65° (and at 105°) hydrogenation gave pipecolic acid and *cis*-4-hydroxypipecolic acid detectable by paper chromatography, and better results were obtained by hydrogenation in water.

4-Benzyloxypicolinic acid (6·46 g.) in water (50 c.c.) was hydrogenated at 105°/70 atm. over Adams catalyst (0·285 g.) for 24 hr. and then filtered. The pale filtrate darkened rapidly and after concentration was again filtered from much dark brown material. The 4-hydroxypipecolic acids were isolated from the soluble mixture (1·91 g.) by preparative paper chromatography on Whatman seed-test paper [32] with the upper phase of the system ethyl acetate–ethanol–acetic acid–water (6 : 1 : 2 : 1). The imino-acid mixture in water (12 c.c.) was placed in a strip (total width 150 cm.), and the upper phase from 300 c.c. of the solvent mixture was used for each 15 cm. Chromatography gave after *ca.* 24 hr. bands of pipecolic acid at 40—48 cm. and the 4-hydroxypipecolic acids at 22—34 cm. Bands were located by heating the paper after spraying it lightly with ninhydrin, and the 4-hydroxypipecolic acids (0·32 g., 8%) were extracted from the appropriate areas with 90% ethanol (Soxhlet), but failed to crystallise. The crude acids were transferred to a ZeoKarb 225 column and elution with 0·1, 0·2, 0·4, and 0·8N-hydrochloric acid (250 c.c. each) gave the 4-hydroxypipecolic acids completely in the last two solvents. A solution of the product (0·29 g.) in dilute hydrochloric acid (charcoal) was concentrated and deposited the (±)-*cis*-4-hydroxypipecolic acid hydrochloride (0·075 g., 1·4%) in prisms, m. p. 253—255° (decomp.), and shown by paper chromatography to contain the *cis*-acid as the only ninhydrin-positive component.

Epimerisation of 4-Hydroxypipecolic Acid and Comparisons with Other Hydroxypipecolic Acids.—(*a*) The *trans*-acid (6 mg.) gave a mixture of *cis*- and *trans*-isomers when heated with N-sodium hydroxide (0·1 c.c.) in a sealed tube at 145° for 9 hr. A trace of the epimer was similarly produced by heating in water alone, but not in N-hydrochloric acid.

[31] Mosher and Look, *J. Org. Chem.*, 1955, **20**, 283.
[32] Brownell, Hamilton, and Casselman, *Analyt. Chem.*, 1957, **29**, 550.

(*b*) The epimeric mixture of imino-acids formed by heating *trans*-4-hydroxypipecolic acid (5 mg.) in saturated aqueous barium hydroxide (0·3 c.c.) in a sealed tube at 155° for 12 hr. was compared with 5-hydroxypipecolic acid obtained from dates,[6] (−)-*cis*-4-hydroxypipecolic acid (provided by Dr. Vanderhaeghe), (±)-*cis*-3-hydroxypipecolic acid,[8] and with Fowden's acid.[2] On paper chromatograms run for 28, 48, and 84 hr. the acids moved as follows: 4-hydroxypipecolic acid from *Acacia* sp. 12·0, 19·1, and 35·5 cm.; 5-hydroxypipecolic acid the same in each case; *cis*-acid from epimerisation 8·7, 16·6, and 31·3 cm., and pipecolic acid 20·8, 32·2 cm. and off the paper. 5-Hydroxypipecolic acid gave a blue-purple colour (and a bright red fluoresence under ultraviolet light) when heated with ninhydrin, and was thus easily distinguished from the natural 4-hydroxy-acid. In phenol–water and an atmosphere containing ammonia and hydrogen cyanide,[33] for 12 and 24 hr. (solvent front moved 27 and 45 cm.), the 4-hydroxy-epimers were not separated and had R_F 0·55 and 0·47; 5-hydroxypipecolic acid R_F 0·53 and 0·45; pipecolic acid R_F 0·33 and 0·27. The synthetic 4-hydroxy-acid was similarly epimerised to a mixture of *cis*- and *trans*-forms indistinguishable from the mixture derived from the natural acid. *cis*-3-Hydroxypipecolic acid (R_F 0·24) was clearly separated from the natural acid and its epimer, and after being heated with barium hydroxide showed several ninhydrin-positive materials all distinguishable from the natural 4-hydroxy-acid and its epimer.

(*c*) (−)-*trans*-*N*-Benzoyl-4-hydroxypipecolic acid (2·49 g.) was heated at 200° for 5 min., then boiled with 6N-hydrochloric acid (100 c.c.) for 6½ hr., and benzoic acid was removed with light petroleum. The aqueous layer was shown by paper chromatography to contain *cis*- and *trans*-4-hydroxypipecolic acid, which after passage through a ZeoKarb 225 column were converted into the copper salts. Crystallisation of these salts gave fractions each shown to contain *cis*- and *trans*-acid in approximately equal quantities (Found, on the first fraction: N, 6·7; CuO, 17·0. Calc. for $C_{12}H_{20}O_6N_2Cu,4H_2O$: N, 6·6; CuO, 18·7%).

(+)-1-*Acetyl*-4-*hydroxy*-D-*pipecolic Lactone* (VII) *and* (+)-cis-4-*Hydroxy*-D-*pipecolic Acid* (VIII).—(−)-*trans*-4-Hydroxy-L-pipecolic acid (2·9 g.) was boiled with acetic acid (30 c.c.) and acetic anhydride (10·2 c.c., 5 equiv.) for 4 hr. The solution was evaporated under reduced pressure, and the brown crystalline residue was dissolved in ethyl acetate (60 c.c.) and decolorised with charcoal before concentration (to 15 g.). Crystallisation at 0° for 18 hr. gave (+)-1-*acetyl*-4-*hydroxy*-D-*pipecolic lactone* in thick, pale brown plates (1·1 g., 32%), m. p. 145° raised to 148—149° by recrystallisation (charcoal) from ethyl acetate, $[\alpha]_D^{24}$ +181° (1% in EtOH), +190° (1% in $CHCl_3$) (Found: C, 57·2; H, 6·6; N, 8·3. $C_8H_{11}O_3N$ requires C, 56·8; H, 6·55; N, 8·3%).

The lactone (1·0 g.) was boiled with 2N-hydrochloric acid (50 c.c.) for 3 hr. before evaporation on a steam-bath. The crystalline residue was dissolved in water (charcoal) and transferred to a ZeoKarb 225 column (28 g.) and the imino-acid was eluted with N-ammonia. Concentration gave a syrup which crystallised, after the addition of ethanol, in prisms (0·74 g., 68%), m. p. 266—269° (decomp.), consisting of (+)-cis-4-*hydroxy*-D-*pipecolic acid dihydrate*, $[\alpha]_D^{24}$ +17° (1% in H_2O), −6·1° (1% in 5N-HCl) (Found: C, 39·8; H, 8·3; N, 8·1; loss on drying at 80° *in vacuo*, 19·3. $C_6H_{11}O_3N,2H_2O$ requires C, 39·8; H, 8·3; N, 7·7; H_2O, 19·9%). The acid was chromatographically indistinguishable from Dr. Vanderhaeghe's (−)-*cis*-acid and from the epimer of the natural *trans*-acid.

L-*Pipecolic Acid and Derivatives*.—L-Pipecolic acid was obtained from *A. excelsa* heartwood in prisms, m. p. 273—275° (decomp.) (lit.,[34] m. p. 274°), $[\alpha]_D^{23}$ −25·2° (2·2% in H_2O) (lit.,[35] −25·2°) (Found: C, 56·2; H, 8·6; N, 11·0. Calc. for $C_6H_{11}O_2N$: C, 55·8; H, 8·6; N, 10·9%). The (−)-hydrochloride, $[\alpha]_D^{22}$ −10·5° (6% in H_2O) (lit.,[35] −5° to −10·3°), $[\alpha]_D^{23}$ −10·5° (2% in 5N-HCl), −10·0° (6% in 5N-HCl), had m. p. 256—258° (decomp.) (lit.,[14] 256° and [36] 258—259°) alone and when mixed with the (±)-hydrochloride. *N*-Benzoyl-L-pipecolic acid crystallised from ethanol–light petroleum, b. p. 60—80°, in prisms, m. p. 133° depressed to 119—122° on admixture with the (±)-benzoyl derivative, and crystallisation from water gave needles, m. p. 132—133°, $[\alpha]_D^{22}$ −72° (1% in EtOH) (lit.,[14] m. p. 145°, $[\alpha]_D$ −72·8°). 1-*Phenylcarbamoyl*-L-*pipecolic acid* (80%) crystallised in prisms, m. p. 178°, from an acidified solution in 5% aqueous sodium carbonate, and had $[\alpha]_D^{20}$ −39° (1% in EtOH) (Found: C, 63·4; H, 6·4; N, 11·4. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·5; N, 11·3%). Recrystallisation from boiling water gave the optically inactive phenylhydantoin in needles, m. p. 159—160°.

[33] Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224.
[34] Grobbelaar, Zacharius, and Steward, *J. Amer. Chem. Soc.*, 1954, **76**, 2912.
[35] Harris and Pollock, *Chem. and Ind.*, 1953, 462.
[36] Zacharius, Thompson, and Steward, *J. Amer. Chem. Soc.*, 1954, **76**, 2908.

1-*Phenylcarbamoyl*-DL-*pipecolic Acid and* 3-*Phenylpiperidino*-(1′,2′:1,5)*hydantoin.*—(±)-Pipecolic acid hydrochloride, m. p. 258—260° (decomp.), was obtained (91%) by hydrogenation of picolinic acid (5 g.) in 5N-hydrochloric acid (20 c.c.) over Adams catalyst (0·2 g.) at 25 atm./60° for 24 hr. This salt (0·66 g.) in N-sodium hydroxide (8 c.c.) was treated with phenyl isocyanate (0·59 g., 1·25 equiv.). Acidification of the filtrate gave crystals of the (±)-1-*phenylcarbamoylpipecolic acid* (0·81 g., 81%), m. p. 138° and 156—158° after resolidification (cyclisation) (Found: C, 63·4; H, 6·8; N, 11·4. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·5; N, 11·3%). Recrystallisation from hot water, after boiling for 1 hr., gave the (±)-*hydantoin* in needles, m. p. 158—159° alone and when mixed with that formed by cyclisation of the (−)-phenylcarbamoyl derivative (Found: C, 67·8; H, 6·2; N, 12·2. $C_{13}H_{14}O_2N_2$ requires C, 67·8; H, 6·1; N, 12·2%).

Ethyl 1-*Ethoxycarbonyl*-3-*oxopyrrolidin*-2-*ylacetate* (X).—Ethyl β-ethoxycarbonylaminopropionate [37] (38·1 g., 0·2 mole) and ethyl fumarate [38] (34·4 g., 0·2 mole) were added successively to a stirred mixture of dry benzene (350 c.c.) and sodium wire (4·6 g., 0·2 g.-atom). Reaction began slowly and the temperature rose to the b. p. during 45 min. and stirring was then discontinued for 30 min. while the last pieces of sodium reacted; and the reddish-brown solution was then boiled and stirred for 30 min. The cooled solution was diluted with ether (100 c.c.) and shaken with ice-water (300 c.c.), and the almost colourless organic phase was extracted with ice-water (2 × 75 c.c.). The combined aqueous phases were extracted with ether (75 c.c.) before being poured into ice (75 g.) and concentrated sulphuric acid (6·5 c.c.), whereupon an oil separated. The strongly acidic solution was saturated with sodium chloride before being extracted with ethyl acetate (3 × 100 c.c.), and the extracts were washed with brine (50 c.c.) containing sodium hydrogen carbonate, and dried (Na_2SO_4). Removal of the ethyl acetate and distillation under reduced pressure gave an oil (53·5 g.) which was dissolved in 10N-hydrochloric acid (150 c.c.). Next day the solution was evaporated under reduced pressure; ethanol (2 × 100 c.c.) was added to the residue, and the mixture evaporated to dryness. The residue was boiled for 4½ hr. with ethanol (150 c.c.) previously saturated with hydrogen chloride, and the solution was then evaporated under reduced pressure; distillation of the residue (37 g.) gave *ethyl* 1-*ethoxycarbonyl*-3-*oxopyrrolidin*-2-*ylacetate* (24·2 g., 50%), b. p. 122—128° (mainly at 125°)/0·3 mm. (Found: C, 53·9; H, 7·0; N, 6·0. $C_{11}H_{17}O_5N$ requires C, 54·3; H, 7·0; N, 5·8%), and a fraction (2·9 g.), b. p. 128—136°/0·3 mm., which yielded the same semicarbazone as the main fraction. Infrared absorption (in Nujol or CCl_4) included carbonyl bands at 5·68, 5·77, and 5·86 μ. The *semicarbazone* prepared at room temperature (7 days) crystallised from ethanol in prisms, m. p. 124° (Found: N, 18·9. $C_{12}H_{20}O_5N_4$ requires N, 18·7%). The dimorphic 2,4-*dinitrophenylhydrazone*, orange plates, m. p. 112—113°, or yellow prisms, m. p. 135°, was obtained in either form by appropriate seeding of a supersaturated ethanolic solution. The lower-melting form sometimes resolidified and then melted again at 133—135° (Found, for the orange form: N, 16·6; for the yellow form: N, 16·4. $C_{17}H_{21}O_8N_5$ requires N, 16·5%).

3-*Hydroxypyrrolidin*-2-*ylacetic Acid* (XI).—Sodium borohydride (0·38 g., 2 equiv.) in water (1 c.c.) was added dropwise during 10 min. to a solution of the above keto-ester (4·86 g.) in ethanol (50 c.c.) initially at 15° and kept below 20° by cooling. After 40 min. 10% sulphuric acid was added until all the borohydride was decomposed. Barium hydroxide octahydrate (19 g.) and water (50 c.c.) were added and the mixture was heated in a boiling-water bath for 16 hr. The mixture was diluted with water (100 c.c.) and filtered when cold. The solution was acidified with 10% sulphuric acid, and the filtrate was continuously extracted with ether for 16 hr. to remove boric acid (0·073 g.). The aqueous solution was basified with aqueous barium hydroxide, and the filtered solution was extracted with ether for 30 hr. before removal of barium as carbonate. Evaporation of the filtrate left a crystalline residue which was digested with ethanol containing a few drops of acetic acid, and the insoluble material (1·11 g., 38%; m. p. 192—198°) was collected; the filtrate contained at least seven ninhydrin-positive substances, and was discarded. The solid appeared to be a single substance and chromatography (19 hr.; solvent front moved 48 cm.) showed a substance with R_F 0·27 (4-hydroxypipecolic acid, R_F 0·20; pipecolic acid, R_F 0·36) which gave a yellow-orange colour with ninhydrin and a white spot with isatin detectable as a non-absorbing area under ultraviolet light. Three crystallisations from aqueous ethanol (charcoal) gave 3-*hydroxypyrrolidin*-2-*ylacetic acid monohydrate* (0·51 g.) in prisms, m. p. 215—216° (decomp.) (Found, on material dried *in vacuo* for 24 hr.: C, 44·5; H,

[37] Braun and Looker, *J. Amer. Chem. Soc.*, 1958, **80**, 359.
[38] Mitchovitch, *Bull. Soc. chim. France*, 1937. **4**, 1661; *Chem. Abs.*, 1938, **32**, 1241.

8·0; N, 8·6. $C_6H_{11}O_3N,H_2O$ requires C, 44·2; H, 8·0; N, 8·6%). The N-(2,4-*dinitrophenyl*) *derivative* (83%) crystallised from aqueous ethanol in prisms, m. p. 205° (Found: C, 45·2; H, 4·6; N, 12·9. $C_{12}H_{13}O_7N_3,\frac{1}{2}H_2O$ requires C, 45·0; H, 4·4; N, 13·1%). The imino-acid was recovered after treatment with nitrous acid under conditions which destroyed added glycine and β-alanine. The phenylcarbamoyl derivative formed from the acid and phenyl isocyanate lost the elements of water to give the *lactone*, which crystallised from water in prisms, m. p. 168°, insoluble in cold aqueous sodium hydrogen carbonate (Found: C, 63·3; H, 5·6; N, 11·4. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%). The lactone was recovered after being heated with 3N-hydrochloric acid on a steam-bath for 8 hr. In Nujol mull it showed NH absorption at 2·95 μ and carbonyl bands at 5·69 (lactone) and 6 μ (urea).

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