949. *Pyrazine Derivatives, Part XIV,* and Aspergillic Acid, Part IV.†*

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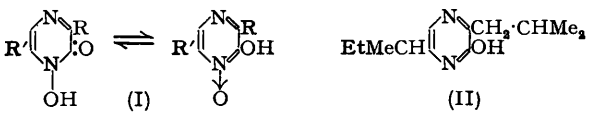
Treatment of DL-leucyl-DL-*iso*leucine anhydride (3-*iso*butyl-6-*sec.*-butyl-2 : 5-diketopiperazine) (V) with phosphoryl chloride gave a mixture from which was isolated 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine (VII), isomeric with deoxyaspergillic acid (2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine) (II). 5-*iso*Butyl-2-*sec.*-butyl-3-hydroxypyrazine (VII) has been synthesised by an unambiguous method.

OF the two structures (I; R = Bu^s, R′ = Bu^i, or *vice versa*) (Dunn, Newbold, and Spring, *J.*, 1949, S 131) for aspergillic acid, a decision was made in favour of the latter by Newbold, Sharp, and Spring (*J.*, 1951, 2679) who synthesised 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (II) and showed it to be identical with racemic deoxyaspergillic acid. The present paper describes an attempt to synthesise deoxyaspergillic acid by a different route. Since with phosphoryl chloride DL-phenylglycine anhydride gives 3-hydroxy-2 : 5-diphenylpyrazine (Gallagher, Newbold, Spring, and Woods, *J.*, 1949, 910) and DL-leucine anhydride gives 2 : 5-di*iso*butyl-3-hydroxypyrazine (Dunn, Newbold, and Spring, *J.*, 1949, 2586), the action of the chloride on DL-leucyl-DL-*iso*leucine anhydride (3-*iso*butyl-6-*sec.*-butyl-2 : 5-diketopiperazine) (V) was investigated.



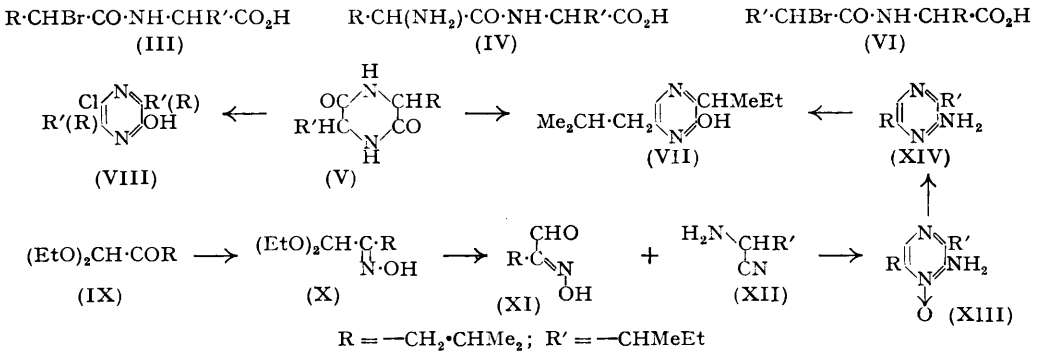
The mixed anhydride has been prepared by two methods (Dunn, Gallagher, Newbold, and Spring, *J.*, 1949, S 126). The first consisted in the dehydration of DL-leucyl-DL-*iso*leucine (IV) by heating it with β-naphthol and gave the diketopiperazine (V), m. p. 275—276°. The second method included the preparation of 2-bromo-4-methylpentanoyl-DL-*iso*leucine (III); this was esterified and the ester was treated with ammonia. The diketopiperazine obtained by this method had m. p. 266—267°. We now find that the intermediate 2-bromo-4-methylpentanoyl-DL-*iso*leucine (III) prepared by the method of Abderhalden, Hirsch, and Schuler (*Ber.*, 1909, **42**, 3394) exists in two racemic forms: one of m. p. 178—179°, identical with that described by Dunn, Gallagher, *et al.* (*loc. cit.*), the other of m. p. 131—133°. Esterification of either form of 2-bromo-4-methylpentanoyl-DL-*iso*leucine followed by treatment of the product with ammonia gave the same product, m. p. 266—267°, fractional crystallisation of which gave a less soluble modification, m. p. 275—276°, of DL-leucyl-DL-*iso*leucine anhydride, identical with that obtained by the dehydration of DL-leucyl-DL-*iso*leucine, and a more soluble modification, m. p. 258—259°.

DL-Leucyl-DL-*iso*leucine anhydride (V) has been prepared by a third route. Condensation of 2-bromo-3-methylpentanoyl chloride with DL-leucine gave two racemic forms of 2-bromo-3-methylpentanoyl-DL-leucine (VI), m. p. 96—98° and 144—145·5° respectively. Treatment of the higher-melting form with ammonia gave the dipeptide DL-*iso*leucyl-DL-leucine. Esterification of each modification of 2-bromo-3-methylpentanoyl-DL-leucine (VI) followed by reaction with ammonia gave a mixture from which the two modifications of DL-leucyl-DL-*iso*leucine anhydride (V) were separated.

Treatment of either form of the anhydride with phosphoryl chloride gave a mixture from which 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine (VII), m. p. 96—98°, was isolated; the isomeric 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (II) (deoxyaspergillic acid) was not isolated. In addition to (VII), 3(or 6)-*iso*butyl-6(or 3)-*sec.*-butyl-2-chloro-5-hydroxypyrazine (VIII) and a mixture of chloropyrazines were isolated. This mixture probably contains 3-*iso*butyl-6-*sec.*-butyl-2 : 5-dichloropyrazine and a monochloro-2-*iso*butyl-5-*sec.*-butylpyrazine but a pure component was not isolated directly from the mixture. Treat-

* Part XIII, *J.*, 1951, 932. † Part III, *J.*, 1951, 2679.

ment of the mixture with sodium ethoxide gave 3(or 6)-*iso*butyl-6(or 3)-*sec.*-butyl-2-chloro-5-ethoxypyrazine, hydrolysed by mineral acid to the 2-chloro-5-hydroxypyrazine (VIII) which was isolated directly from the original reaction mixture.



The structure ascribed to the hydroxypyrazine (VII) depended on the argument that its method of formation requires it to be either 5-*iso*butyl-2-*sec.*-butyl- or 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine. Of these, the latter (deoxyaspergillic acid) has been unambiguously synthesised (Newbold, Sharp, and Spring, *loc. cit.*) and differs from (VII). This has been confirmed by the following synthesis. 2-Keto-4-methylpentanal diethyl acetal (IX) (Dakin and Dudley, *J.*, 1914, **105**, 2453) was converted into its oxime (X) which on controlled hydrolysis gave the α-oximino-aldehyde (XI). Condensation of the last compound with DL-*iso*leucine nitrile (XII) gave 2-amino-6-*iso*butyl-3-*sec.*-butylpyrazine 1-oxide (XIII) which on dithionite reduction and subsequent treatment with nitrous acid gave 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine (VII) identical with the product from the diketopiperazine.

Experimental

2-Bromo-4-methylpentanoyl-DL-*iso*leucine was prepared as described by Dunn, Gallagher, Newbold, and Spring (*loc. cit.*). The crude product, m. p. 134—144°, after six crystallisations from aqueous ethanol gave the higher-melting racemate as rhombic plates, m. p. 178—179° (38%). The more soluble *isomer* was obtained from the mother-liquors as plates, m. p. 131—133° (50%) (Found: C, 46·8; H, 7·3; N, 4·5. $C_{12}H_{22}O_3NBr$ requires C, 46·8; H, 7·2; N, 4·5%).

2-*Bromo*-3-*methylpentanoyl*-DL-*leucine*.—2-Bromo-3-methylpentanoyl chloride (40 g.; Abderhalden, Hirsch, and Schuler, *loc. cit.*) and N-sodium hydroxide (267 c.c.) were added simultaneously during 45 minutes with vigorous stirring and ice-cooling to DL-leucine (20 g.) in N-sodium hydroxide (153 c.c.). The solution was acidified with 5N-hydrochloric acid (54 c.c.), an oil separating which rapidly solidified (42·7 g.; m. p. 115—125°). The solid (26 g.) was fractionally crystallised from benzene, to give a less soluble fraction A and a more soluble fraction B. Repeated crystallisation of fraction A from benzene gave 2-*bromo*-3-*methylpentanoyl*-DL-*leucine* as rhombic plates (6·2 g.), m. p. 144—145·5° (Found: C, 46·7; H, 7·0; N, 4·5. $C_{12}H_{22}O_3NBr$ requires C, 46·8; H, 7·2; N, 4·5%). After many crystallisations from light petroleum (b. p. 60—80°), fraction B gave the second *form* (10·5 g.) as rods, m. p. 96—98° (Found: C, 46·9; H, 7·2; N, 4·5%).

DL-iso*Leucyl*-DL-*leucine*.—A solution of 2-bromo-3-methylpentanoyl-DL-leucine (m. p. 142—144°) in aqueous ammonia (*d* 0·88) was heated at 140° for 1 hour, then evaporated under reduced pressure, and the residue was extracted with boiling acetone. The extract was concentrated and kept at 0° overnight. The solid which separated was washed with cold ethyl acetate and crystallised thrice from water, to give DL-iso*leucyl*-DL-*leucine* (20%) as prisms, m. p. 272—274° (Found: C, 58·6; H, 9·9; N, 11·7. $C_{12}H_{24}O_3N_2$ requires C, 59·0; H, 9·9; N, 11·5%), insoluble in cold water, ether, or benzene, and slightly soluble in cold acetone or ethanol.

DL-*Leucyl*-DL-iso*leucine Anhydride* (3-iso*Butyl*-6-sec.-*butyl*-2 : 5-*diketopiperazine*).—(*a*) 2-Bromo-4-methylpentanoyl-DL-*iso*leucine (12 g.; m. p. 131—133°) was esterified and the ester treated with alcoholic ammonia as described for the higher-melting modification (Dunn, Gal-

lagher, *et al.*, *loc. cit.*). The solid separating from the final reaction mixture crystallised from ethyl acetate, to yield DL-leucyl-DL-*iso*leucine anhydride as needles, m. p. 265—267°. Fractional crystallisation from ethanol gave a less soluble modification as needles, m. p. 275—277° (0·75 g.) undepressed when mixed with the diketopiperazine obtained by Dunn, Gallagher, *et al.* (*loc. cit.*) by treatment of DL-leucyl-DL-*iso*leucine with β-naphthol (Found: N, 12·3. Calc. for $C_{12}H_{22}O_2N_2$: N, 12·4%), and a more soluble *form* (3·4 g.) which separated as needles, m. p. 258—259° (Found: N, 12·5. $C_{12}H_{22}O_2N_2$ requires N, 12·4%). A mixture of the two modifications has an intermediate m. p.

Treatment of the higher-melting form of 2-bromo-4-methylpentanoyl-DL-*iso*leucine (12·0 g.) as described above gave a DL-leucyl-DL-*iso*leucine anhydride separating as needles, m. p. 265—267°, from ethyl acetate. Fractionation from ethanol gave the modification, m. p. 275—277° (2·1 g.), as needles, undepressed in m. p. when mixed with the specimen described above (Found: C, 63·9; H, 10·0; N, 12·2. Calc. for $C_{12}H_{22}O_2N_2$: C, 63·7; H, 9·8; N, 12·4%), and the more soluble *modification* separating as needles (2·1 g.) (from ethanol), m. p. 258—259°, undepressed when mixed with the lower-melting form of the diketopiperazine described above (Found: C, 63·4; H, 9·9; N, 12·6%).

(*b*) A solution of 2-bromo-3-methylpentanoyl-DL-leucine (m. p. 142—144°) in dry ethanol was saturated with dry hydrogen chloride and heated under reflux for 3 hours. The mixture was evaporated under reduced pressure. The residue crystallised from light petroleum (b. p. 60—80°) to give the *ethyl* ester which after four recrystallisations had m. p. 85·5—86·5° (Found: C, 49·9; H, 8·0; N, 4·5. $C_{14}H_{26}O_3NBr$ requires C, 50·0; H, 7·8; N, 4·2%). The crude ester, treated with ethanolic ammonia as described under (*a*), gave the two forms of DL-leucyl-DL-*iso*leucine anhydride which were separated by fractionation from ethanol, the more soluble form separating as needles, m. p. and mixed m. p. 259—260°, and the less soluble form separating as needles, m. p. and mixed m. p. 275—276°.

Similar treatment of the modification of m. p. 96—98° of 2-bromo-3-methylpentanoyl-DL-leucine gave a crude diketopiperazine which when fractionated from ethanol gave the higher-melting modification of DL-leucyl-DL-*iso*leucine as needles, m. p. and mixed m. p. 275—277° (Found: N, 12·3%), and the more soluble modification as needles, m. p. and mixed m. p. 259—260° (Found: N, 12·1%).

5-iso*Butyl*-2-sec.-*butyl*-3-*hydroxypyrazine* (see also p. 4874).—DL-Leucyl-DL-*iso*leucine anhydride (m. p. 275—276° or m. p. 259—260°; 8·9 g.) was treated with phosphoryl chloride (90 c.c.) and the mixture refluxed for 2 hours. The excess of chloride was removed under reduced pressure and the residue triturated with ice-water. The mixture was neutralised with 3N-potassium hydroxide (litmus) and extracted with ether. The ethereal solution was extracted with 3N-potassium hydroxide (extract A) and then with 6N-hydrochloric acid (extract B) and dried (Na_2SO_4) (solution C). Extract B was made just alkaline with 3N-potassium hydroxide and extracted with ether and the extract dried (Na_2SO_4). Removal of the ether and distillation of the residue gave a colourless oil, b. p. 80°/1 mm., n_D^{16} 1·4845 (Found: C, 74·7; H, 10·2; N, 14·6, 14·7%). Extract A was neutralised with hydrochloric acid (*d* 1·16) (litmus) and extracted with ether. The ethereal solution was extracted with hydrochloric acid (2N) (ethereal solution D), the aqueous phase neutralised (litmus) with 3N-potassium hydroxide, and the solid (1·04 g.; m. p. 76—80°) collected. Sublimation at 95°/2 × 10^{-3} mm. followed by crystallisation from aqueous ethanol gave 5-iso*butyl*-2-sec.-*butyl*-3-*hydroxypyrazine* as needles, m. p. 97—98° (Found: C, 69·4; H, 9·3; N, 13·3. $C_{12}H_{20}ON_2$ requires C, 69·2; H, 9·7; N, 13·45%). Light absorption in ethanol: Max. at 229 (ε = 7000) and 325 mμ (ε = 7600). A mixture of 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine with racemic deoxyaspergillic acid, m. p. 103—104° had m. p. 90—92°. The hydroxypyrazine is readily soluble in organic solvents; it is insoluble in cold and only slightly soluble in hot water but it is readily soluble in 3N-potassium hydroxide or 2N-hydrochloric acid. The 6-*phenylazo*-derivative, prepared as described for that of racemic deoxyaspergillic acid by Dunn, Gallagher, *et al.* (*loc. cit.*), separated from aqueous ethanol as orange-red needles, m. p. 203—205° (decomp.) (Found: C, 69·2; H, 7·7; N, 18·3. $C_{18}H_{24}ON_4$ requires C, 69·2; H, 7·75; N, 17·9%). A mixture with phenylazodeoxyaspergillic acid, m. p. 188—189°, had m. p. 189—191°.

5-*Bromo*-6-iso*butyl*-3-sec.-*butyl*-2-*hydroxypyrazine*.—5-*iso*Butyl-2-*sec.*-butyl-3-hydroxypyrazine (0·5 g.) in acetic acid (10 c.c.) and water (6 c.c.) was treated with bromine (0·4 g.) in acetic acid (1 c.c.). The crystalline precipitate was recrystallised from aqueous ethanol from which the *bromo*-derivative separated as needles, m. p. 150—151° (Found: C, 50·1; H, 6·9; N, 9·3. $C_{12}H_{19}ON_2Br$ requires C, 50·2; H, 6·7; N, 9·8%). Light absorption in ethanol: Max. at 233 (ε = 9700) and 331 mμ (ε = 7100).

3(*or* 6)-iso*Butyl*-6(*or* 3)-sec.-*butyl*-2-*chloro*-5-*ethoxypyrazine*.—Evaporation of the ethereal solution C, followed by distillation of the oily residue, gave a colourless oil (5·0 g.), b. p. 102—104°/2 mm., n_D^{15} 1·5120 (Found : C, 58·8; H, 7·5; N, 12·6; Cl, 25·9%). Redistillation gave a fraction, b. p. 105°/1 mm., n_D^{16} 1·5155 (Found : C, 57·4; H, 7·5. Calc. for $C_{12}H_{18}N_2Cl_2$: C, 55·2; H, 6·9. Calc. for $C_{12}H_{19}N_2Cl$: C, 63·6; H, 8·3%), and a fraction, b. p. 85°/0·5 mm., n_D^{16} 1·4995 (Found : C, 65·9; H, 8·8. Calc. for $C_{12}H_{19}N_2Cl$: C, 63·6; H, 8·3. Calc. for $C_{12}H_{20}N_2$: C, 74·95; H, 10·5%). The oil, b. p. 102—104°/2 mm. (3·95 g.), was heated at 110° for 4 hours with ethanolic sodium ethoxide (4·8% ; 25 c.c.). The ethanol was removed under reduced pressure, the residue diluted with water, and the mixture neutralised (litmus) with hydrochloric acid (*d* 1·16) and extracted with ether. The ethereal extract was evaporated and the residue heated under reflux with hydrochloric acid (100 c.c. ; 5N) for 18 hours. The mixture was extracted with ether, and the ethereal extract dried (Na_2SO_4) and evaporated. The residue was distilled, to give 3(*or* 6)-iso*butyl*-6(*or* 3)-sec.-*butyl*-2-*chloro*-5-*ethoxypyrazine* (2·15 g.) as a colourless oil, b. p. 115°/1 mm., n_D^{13} 1·4995 (Found : C, 62·1; H, 8·6; N, 10·8. $C_{14}H_{23}ON_2Cl$ requires C, 62·1; H, 8·6; N, 10·3%).

3(*or* 6)-iso*Butyl*-6(*or* 3)-sec.-*butyl*-2-*chloro*-5-*hydroxypyrazine*.—(*a*) A solution of the chloroethoxypyrazine in aqueous ethanol (65%) was saturated with hydrogen chloride and heated under reflux for 5 hours, then evaporated under reduced pressure, the residue was diluted with water (20 c.c.) and the product isolated by means of ether. Recrystallisation from aqueous ethanol gave 3(or 6)-iso*butyl*-6(or 3)-sec.-*butyl*-2-*chloro*-5-*hydroxypyrazine* as needles, m. p. 139—140° (30%) (Found : C, 58·8; H, 8·0; N, 11·3. $C_{12}H_{19}ON_2Cl$ requires C, 59·4; H, 7·9; N, 11·5%). Light absorption in ethanol : Max. at 232 (ε = 8000) and 324 mμ (ε = 6000). The compound is soluble in the common organic solvents and in 3N-potassium hydroxide but insoluble in water or 2N-hydrochloric acid.

(*b*) The ethereal solution D (above) was extracted with 3N-potassium hydroxide. The alkaline extract was neutralised (litmus) with hydrochloric acid (*d* 1·16) and kept at 0° for 1 hour. The solid (50 mg.) was collected, sublimed at 95°/2 × 10^{-3} mm., and crystallised from aqueous ethanol, to give the chloro-hydroxypyrazine as needles, m. p. 138—139°, undepressed when mixed with the specimen prepared by method (*a*) (Found : N, 11·9%).

4-*Methyl*-2-*oximinopentanal Diethyl Acetal*.—2-Keto-4-methylpentanal diethyl acetal (14·6 g. ; Dakin and Dudley, *loc. cit.*) was stirred for 8 hours at room temperature with hydroxylamine hydrochloride (5·5 g.) and sodium carbonate (5·75 g.) in water (70 c.c.) and methanol (110 c.c.). Next morning, the mixture was worked up as described for the 3-methyl isomer (Newbold, Sharp, and Spring, *loc. cit.*), to give 4-*methyl*-2-*oximinopentanal diethyl acetal* (14·0 g.) as a colourless oil, b. p. 102—106°/2 mm., n_D^{18} 1·4430 (Found : C, 59·1; H, 9·85. $C_{10}H_{21}O_3N$ requires C, 59·1; H, 10·4%).

4-*Methyl*-2-*oximinopentanal*.—The diethyl acetal (5·7 g.) was hydrolysed in methanol (35 c.c.) and buffer solution (35 c.c. ; pH 3·5) following the procedure described for the 3-methyl isomer (*loc. cit.*), to give the *aldehyde* (2·3 g.) as a pale yellow viscous oil, b. p. 54—60°/0·5 mm., n_D^{18} 1·4713 (Found : C, 56·1; H, 8·2. $C_6H_{11}O_2N$ requires C, 55·8; H, 8·6%).

DL-iso*Leucine Nitrile Hydrochloride*.—By using the method described in the preparation of DL-leucine nitrile hydrochloride (*loc. cit.*) and crystallisation from acetone, 2-methylbutanal gave DL-iso*leucine nitrile hydrochloride* as plates, m. p. 190—195° (decomp.) (Found : C, 49·1; H, 8·8; N, 18·4; Cl, 24·0. $C_6H_{13}N_2Cl$ requires C, 48·5; H, 8·8; N, 18·8; Cl, 23·9%). The *flavianate* separated from ethanol or propanol–light petroleum (b. p. 60—80°) as yellow needles, m. p. 200—210° (decomp.) on rapid heating; it chars without melting on slow heating (Found : C, 45·3; H, 4·2. $C_6H_{12}N_2,C_{10}H_6O_8N_2S$ requires C, 45·1; H, 4·2%).

2-*Amino*-6-iso*butyl*-3-sec.-*butylpyrazine* 1-*Oxide*.—*iso*Leucine nitrile hydrochloride (2·50 g.) and 4-methyl-2-oximinopentanal (2·17 g.) were heated together for 4 hours in dry chloroform (14 c.c.) with 4-methylmorpholine (1·86 c.c.) as described for 2-amino-3-*iso*butyl-6-*sec.*-butylpyrazine 1-oxide (*loc. cit.*). The crude mixture, after removal of the chloroform, was shaken with ether and water. The ethereal solution was extracted with hydrochloric acid (3N), the aqueous acid solution made alkaline with sodium hydroxide, and the viscous oil (0·85 g.) which separated isolated by means of ether. The oil solidified. Crystallisation from light petroleum (b. p. 60—80°) gave 2-*amino*-6-iso*butyl*-3-sec.-*butylpyrazine* 1-*oxide* as prisms, m. p. 84—86°, sintering at 80—84° (Found : C, 64·5; H, 9·7. $C_{12}H_{21}ON_3$ requires C, 64·5; H, 9·5%). It gives an intense blue colour in ethanol with ferric chloride, the colour being discharged with dilute hydrochloric acid.

5-iso*Butyl*-2-sec.-*butyl*-3-*hydroxypyrazine*.—The foregoing oxide (1·87 g.) was reduced with sodium dithionite (42 g.) in aqueous ethanol as described for 2-amino-3-*iso*butyl-6-*sec.*-butyl-

pyrazine (*loc. cit.*), but with heating for 60 hours. Crude 3-amino-5-*iso*butyl-2-*sec.*-butylpyrazine was obtained as an amber oil (0·60 g.) which gave only a slight blue colour in ethanol with ferric chloride. The oil (0·48 g.) was converted into 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine by reaction with nitrous acid as described for 2-*iso*butyl-5-*sec.*-butyl-3-hydroxypyrazine (*loc. cit.*). The solid (80 mg.; m. p. 93—94°) precipitated from the sodium hydroxide solution by means of hydrochloric acid, was sublimed at 110—120°/10^{-3} mm. and crystallised thrice from aqueous ethanol, to give 5-*iso*butyl-2-*sec.*-butyl-3-hydroxypyrazine as needles m. p. 96—97° (Found: C, 68·7; H, 9·5%) alone or mixed with the sample obtained as on p. 4872. A mixture with racemic deoxyaspergillic acid (m. p. 103—104°) had m. p. 85—91°.

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