SYNTHESIS OF 4-(N-ACETYL-L-TYROSYL)AMINO-2-IODOBENZOIC ACID

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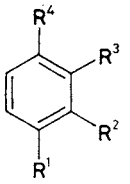
The title compound was prepared by a three-step synthesis from diacetyltyrosine (*XV*) and 4-amino-2-iodobenzoic acid (*I*). Syntheses of acid *I* and 4-amino-3-iodobenzoic acid (*II*) have been revised and modified. ^{1}H and ^{13}C NMR spectra of the synthesized compounds are discussed.

4-(N-Acetyl-L-tyrosyl)aminobenzoic acid[1,2] (*XI*) is used clinically for examination of pancreatic function. On administration, it is specifically cleaved with the pancreatic enzyme chymotrypsin under formation of 4-acetylaminobenzoic acid which is excreted in urine. In order to follow this process it would be useful to insert radioactive iodine atom into the molecule of 4-aminobenzoic acid which is practically not metabolized[1]. The present study concerns the synthesis of 4-(N-acetyl-L-tyrosyl)-amino-2-iodobenzoic acid (*XIII*) in which the labelling with radioactive iodine can be realized by isotopic exchange directly before application.

The syntheses of 2-iodo- and 3-iodo-4-aminobenzoic acids (*I* and *II*, respectively) have already been described[3,4]. When reproducing the described synthesis of the acid *I* we obtained results different from those reported. Mercuration and iodination of 4-aminobenzoic acid which should lead[5] to the desired acid *I* gave 4-amino-3-iodobenzoic acid (*II*) as the principal product in 40% yield (the position of the iodine atom has been proven by ^{1}H and ^{13}C NMR spectroscopy, vide infra).

We therefore started from 4-nitrotoluene (*III*) which was iodinated in the presence of silver sulfate[6]. The resulting 2-iodo-4-nitrotoluene (*IV*; yield 61%) was reduced with tin(II) chloride in hydrochloric acid[6], affording 4-amino-2-iodotoluene (*V*) in 65% yield. This compound was acetylated and the resulting 4-acetylamino-2-iodotoluene (*VI*) was oxidized with potassium permanganate[3] to give 4-acetylamino-2-iodobenzoic acid (*VII*) in 22% yield. Hydrolysis by boiling with hydrochloric acid[3] furnished 4-amino-2-iodobenzoic acid (*I*; 70%).



I, R^1= COOH ; R^2= I ; R^3= H ; R^4= NH_2
II, R^1= COOH ; R^2= H ; R^3= I ; R^4= NH_2
III, R^1= CH_3 ; R^2= H ; R^3= H ; R^4= NO_2
IV, R^1= CH_3 ; R^2= I ; R^3= H ; R^4= NO_2
V, R^1= CH_3 ; R^2= I ; R^3= H ; R^4= NH_2
VI, R^1= CH_3 ; R^2= I ; R^3= H ; R^4= $NHCOCH_3$
VII, R^1= COOH ; R^2= I ; R^3= H ; R^4= $NHCOCH_3$
VIII, R^1= COOH ; R^2= I ; R^3= H ; R^4= NO_2
IX, R^1= COOH ; R^2= IO ; R^3= H ; R^4= NO_2
X, R^1= COOH ; R^2= H ; R^3= H ; R^4= NH_2

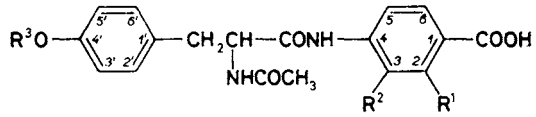
Since the yields in the described reaction sequence were not satisfactory, 2-iodo-4-nitrotoluene (*IV*) was oxidized[7] with alkaline potassium permanganate. This modification gave 2-iodo-4-nitrobenzoic acid (*VIII*) in 33% yield. Oxidation of compound *IV* with potassium permanganate under phase-transfer conditions[8] with cetyltrimethylammonium bromide in water, water–tetrachloromethane or water–chlorobenzene (in both solvent mixtures with or without magnesium sulfate) afforded only negligible amounts of 2-iodo-4-nitrobenzoic acid (*VIII*) and up to 90% of the recovered starting compound. A modified oxidation of *IV* with chromium trioxide[9] in a mixture of acetic acid, acetic anhydride and sulfuric acid proved to be the method of choice, affording in 60% yield 2-iodoso-4-nitrobenzoic acid (*IX*) (1-hydroxy-6-nitro-1,2-benziodoxolin-3-one). The compound *IX* was then reduced with potassium iodide in acetic acid to give 2-iodo-4-nitrobenzoic acid (*VIII*) in 75% yield. Reduction of *VIII* with ferrous sulfate in the presence of ammonia[10] or barium hydroxide[11], or with sodium dithionate, was unsuccessful. Finally, the reduction was achieved with tin(II) chloride in acetic acid, the desired 4-amino-2-iodobenzoic acid (*I*) being obtained in 67% yield.

The structure of the acids *I* and *II* was assigned unequivocally on the basis of their ^1H and ^{13}C NMR spectra. Since formally both the compounds form the same spin systems, they were distinguished by comparison of the observed chemical shifts with the values calculated for the structures *I* and *II*. For the calculation we used the published NMR data for *p*-aminobenzoic acid (^1H NMR: refs[13,14]; ^{13}C NMR: ref.[15], both in hexadeuterodimethyl sulfoxide) and the values of iodine-induced effects in the given positions of the aromatic nucleus[16]. Although the agreement between the calculated and found δ-values was far from excellent (which is not very surprising for trisubstituted benzenes, moreover with the sterically very bulky iodine

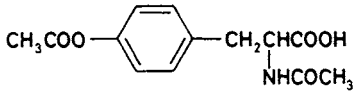
atom), it was nevertheless sufficent for distinction between *I* and *II* (Table I). Already the chemical shift of the "isolated" proton between the two substituents is characteristic: (*I*: δ 7·20, *II*: δ 8·10). Since carboxyl and iodine cause a marked downfield shift of the *ortho*-proton signal (0·85 and 0·39 ppm, respectively) whereas the amino group has an opposite effect (−0·75 ppm), it is obvious that the proton signal at δ 8·10 must be due to a proton in the *ortho*-position to the carboxyl and iodine and that the corresponding compound has the structure *II*.

N,O-Diacetyl-L-tyrosine (*XV*) was prepared by acetylation of L-tyrosine[2] and was further converted in situ into the mixed anhydride by reaction with chloroformate. Subsequent condensation with the acid *I* afforded 4-(N,O-diacetyl-L-tyrosyl)amino--2-iodobenzoic acid (*XII*) in good yield. The same experiment with the 3-iodo acid *II* gave no condensation product *XIV*, even after several trials, the only isolated material being the starting compound.

Alkaline hydrolysis of *XII* at room temperature afforded the desired 4-(N-acetyl-L--tyrosyl)amino-2-iodobenzoic acid (*XIII*).



XI, $R^1 = R^2 = R^3 = H$
XII, $R^1 = I$; $R^2 = H$; $R^3 = COCH_3$
XIII, $R^1 = I$; $R^2 = H$; $R^3 = H$
XIV, $R^1 = H$; $R^2 = I$; $R^3 = H$



XV

EXPERIMENTAL

The melting points were determined on a Boetius (G.D.R.) block and are uncorrected. Optical rotations were measured on an automatic polarimeter ETL-NPL (Bendix–Ericsson) in methanol; accuracy $\pm 2°$. IR spectra were recorded in Nujol on a PE 684 (Perkin–Elmer) spectrometer. ^1H and ^{13}C NMR spectra were obtained with a Varian XL 200 instrument (^1H 200·057 MHz, ^{13}C 50·309 MHz) in hexadeuterodimetyl sulfoxide, using hexamethyldisiloxane (^1H NMR) and dimethyl sulfoxide (^{13}C NMR) as internal standards. All chemical shifts are given in ppm (δ-scale). The coupling constants were obtained by analysis of the first order. Mass spectra were measured on a Varian MAT 311 spectrometer (ionizing electron energy 70 eV, ion source temperature 200°C, ion current 1 mA, direct inlet at 100—200°C). Thin-layer chromatography (TLC)

was done on Silufol sheets (Kavalier, Votice, Czechoslovakia); detection by UV light or spraying with 5% phosphomolybdic acid and heating. Column chromatography was performed on silica gel Silpearl (Kavalier, Votice). Analytical samples were dried in vacuo over phosphorus pentoxide for 12 h.

2-Iodo-4-nitrotoluene (IV): b.p. 94–96°C/13·3 Pa, m.p. 49–51°C (reported[6] m.p. 53–54°C); *4-amino-2-iodotoluene* (V): m.p. 37–38°C (reported[12] m.p. 39°C); *4-acetylamino-2-iodotoluene* (VI): m.p. 128–130°C (reported[12] m.p. 130°C); *4-acetylamino-2-iodobenzoic acid* (VII): m.p. 217–219°C (reported[3] m.p. 213–214°C); N,O-*diacetyl*-L-*tyrosine* (XV): m.p. 168·5–171°C (reported[2] m.p. 168–169°C).

Iodination (via Mercuration) of 4-Aminobenzoic Acid (*X*)

A solution of mercuric acetate (140 g) in water (600 ml) was added to a boiling solution of 4-aminobenzoic acid (*X*; 60·0 g) in water (2 l). A solution of sodium carbonate (44·5 g) in water (400 ml) was added during 4 h to the stirred refluxing above-mentioned mixture. The reaction mixture was then stirred and refluxed for 5 h. The separated organomercurial derivate was filtered, boiled with water (1·5 l), filtered, suspended in hot water (500 ml) and converted into the sodium salt with sodium hydroxide (35·0 g) in water (200 ml). The insoluble residue was filtered and the filtrate was acidified with conc. hydrochloric acid (75 ml). The white precipitate was filtered, washed with hot water (500 ml) and dried at 90°C; yield 98·0 g (67%) of the organomercurial compound.

A solution of iodine (58·4 g) in ethanol (1 l) was added to a suspension of the organomercury derivative (80·0 g) in water (100 ml). After stirring and refluxing for 3 h, the mixture was cooled filtered and the material on filter was washed with ethanol (250 ml). The solvent was evaporated under diminished pressure, the dry residue was triturated with 20% solution of potassium iodide (500 ml), filtered and washed with water. The crude product was dissolved in a solution of sodium carbonate (10%; 500 ml), decolorized with charcoal and acidified with hydrochloric acid. The precipitated product was collected and crystallized from aqueous ethanol to afford 4-amino-3-iodobenzoic acid (*II*; 25·1 g; 40%), m.p. 206–209°C (chloroform–methanol) (reported[5] m.p. 200–201°C). For $C_7H_6INO_2$ (262·9) calculated: 31·96% C, 2·30% H, 48·24% I, 5·32% N; found: 31·81% C, 2·50% H, 47·46% I, 5·41% N. IR spectrum (cm^{-1}): 3 449, 3 353 (NH_2), 1 655 (COOH). Mass spectrum, *m/z* (%): 263 (100), 245 (45), 235 (3), 217 (10), 136 (12). ^1H NMR: 5·94 bs, 2 H (NH_2); 6·74 d, 1 H (H-5, $J(5, 6) = 8·4$); 7·65 dd, 1 H (H-6, $J(6, 5) = 8·4$, $J(6, 2) = 1·9$); 8·10 d,

TABLE I

Comparison of found and calculated ^1H and ^{13}C chemical shifts for compounds *I* and *II*

Compound	H-2	H-3	H-5	H-6	C-1	C-2	C-3	C-4	C-5	C-6
I (obs.)	—	7·20	6·55	7·62	128·7	106·6	134·9	162·4	121·8	142·3
I (calc.)	—	6·92	6·53	7·50	128·6	100·1	123·6	156·7	113·3	135·0
II (obs.)	8·10	—	6·74	7·65	119·6	140·7	81·3	152·8	113·1	130·9
II (calc.)	8·10	—	6·32	7·71	121·3	142·3	81·4	164·0	116·3	132·0

1 H (H-2, $J(2, 6) = 1{\cdot}9$); 12·27 bs, 1 H (COOH). ^{13}C NMR: 81·3 s (C-3); 113·1 d (C-5); 119·6 s (C-1); 130·9 d (C-6); 140·7 d (C-2); 152·8 s (C-4); 166·3 s (COOH).

4-Amino-2-iodobenzoic Acid (*I*)

A mixture of 4-acetylamino-2-iodobenzoic acid (*VII*; 8·0 g) and conc. hydrochloric acid (40 ml) was refluxed for 1·5 h. The reaction mixture was diluted with water (200 ml) and evaporated. The dry residue was dissolved in warm water (250 ml) and treated with charcoal. Repeated crystallization from water afforded the title acid *I* (4·3 g; 71%), m.p. 191—194°C (decomp.) (reported[17] m.p. 188°C (decomp.), and m.p. 180°C (ref.[3])). IR spectrum (cm^{-1}): 3 447, 3 356 (NH_2), 1 655 (COOH). Mass spectrum, *m/z* (%): 263 (100), 245 (30), 217 (25), 136 (31), 127 (32). ^{1}H NMR: 5·96 bs, 2 H (NH_2); 6·55 dd, 1 H (H-5, $J(5, 6) = 8{\cdot}5$; $J(5, 3) = 2{\cdot}3$); 7·20 d, 1 H (H-3, $J(3, 5) = 2{\cdot}3$); 7·62 d, 1 H (H-6, $J(6, 5) = 8{\cdot}5$); 12·24 bs, 1 H (COOH). ^{13}C NMR: 106·6 s (C-2); 121·8 d (C-5); 128·7 s (C-1); 134·9 d (C-3); 142·3 d (C-6); 162·4 s (C-4); 175·9 s (COOH). For $C_7H_6INO_2$ (262·9) calculated: 31·96% C, 2·30% H, 48·24% I, 5·32% N; found: 31·71% C, 2·60% H, 47·97% I, 5·50% N.

2-Iodo-4-nitrobenzoic Acid (*VIII*)

A) A solution of potassium permanganate (65 g) in water (2 l) was added during 5·5 h to a vigorously stirred refluxing suspension of 2-iodo-4-nitrotoluene (*IV*; 30·0 g) in 0·1M-NaOH (440 ml). After stirring and refluxing for further 1 h, methanol (20 ml) was added, the hot mixture was filtered, acidified with dilute hydrochloric acid and cooled. The separated crude product was crystallized from water to give 2-iodo-4-nitrobenzoic acid (*VIII*; 10·7 g; 33%), m.p. 144 to 146°C (reported[3] m.p. 142°C).

B) Concentrated sulfuric acid (100 ml) was added at room temperature to a stirred solution of 2-iodo-4-nitrotoluene (*IV*; 25·4 g) in acetic acid (250 ml) and acetic anhydride (250 ml). After cooling to 5°C, chromium trioxide (90·0 g) was added at this temperature during 4 h. The mixture was then stirred at 15°C for further 4 h and at room temperature overnight, and poured on ice (1·5 kg). Sodium chloride (30·0 g) was added and, after standing for 4 h, the yellow precipitate was filtered, washed with water, dissolved in 10% solution of potassium carbonate (500 ml) and filtered. Acidification to pH 6 and cooling afforded the product which was repeatedly crystallized from 90% acetic acid. The obtained 2-iodoso-4-nitrobenzoic acid (*IX*; 18·1 g; 60·6%) melted at 188—195°C with decomposition (reported[3] m.p. 190—201°C). ^{1}H NMR: 8·14 d, 1 H (H-6, $J(6, 5) = 9{\cdot}9$); 8·49 m, 2 H (H-3, H-5). ^{13}C NMR: 121·9 d (C-5); 122·3 s (C-2); 126·0 d (C-3); 132·2 d (C-6); 136·8 s (C-1); 151·6 s (C-4); 166·2 s (COOH).
A solution of 2-iodoso-4-nitrobenzoic acid (*IX*; 9·6 g) and potassium iodide (13·0 g) in 5% acetic acid (110 ml) was stirred at room temperature for 1 h (immediate separation of iodine). The separated iodine was reduced to iodide by introduction of sulfur dioxide, the mixture was cooled, the desired acid *VIII* was collected and crystallized from water; yield 6·0 g (73%), m.p. 145—146°C.

Reduction of 2-Iodo-4-nitrobenzoic Acid (*VIII*)

Tin dichloride (1·8 g of dihydrate) was added to a solution of acid *VIII* (2·0 g) in acetic acid (15 ml), the mixture was stirred at 80—90°C for 1 h, diluted with hot water (20 ml), saturated with hydrogen sulfide and filtered. The solid on the filter was washed with hot water (50 ml) and the combined filtrates were taken down in vacuo. The dry residue (2·6 g) was dissolved in ethanol (50 ml) and the solution was filtered through a short column of charcoal and silica gel. The ethanol was evaporated, the residue was dissolved in 5% sodium hydroxide solution (50 ml),

extracted twice with ether (50 ml) and the aqueous phase was filtered through a column of charcoal and silica gel. Acidification with conc. hydrochloric acid afforded the acid *I* which was crystallized from aqueous ethanol; yield 1·2 g (67%), m.p. 190—193°C (decomp.).

4-(N,O-Diacetyl-L-tyrosyl)amino-2-iodobenzoic Acid (*XII*)

N-Ethylpiperidine (1·15 ml), followed by ethyl chloroformate (0·8 ml), was added under stirring and cooling to a solution of N,O-diacetyl-L-tyrosine (*XV*; 2·29 g) in anhydrous tetrahydrofuran (26 ml), precooled to −10°C. After 15 min a solution of 4-amino-2-iodobenzoic acid (*I*; 2·27 g) in tetrahydrofuran (5 ml) and a solution of *p*-toluenesulfonic acid (0·17 g) in tetrahydrofuran (1·2 ml) were added simultaneously. The mixture was stirred for further 30 min at −10°C and then for 3·5 h at room temperature. Water (200 ml) was added and the mixture was set aside for 2 days. The solid was filtered, washed with water and dried. The crude product (3·32 g) was extracted with boiling ether (50 ml) and acetone (20 ml). The solid residue was crystallized from ethanol, affording the diacetyl derivative *XII* (1·9 g; 42%), m.p. 247—249°C (decomp.); $[\alpha]_D$ +5° (*c* 0·42). For $C_{20}H_{19}IN_2O_6$ (509·9) calculated: 47·05% C, 3·72% H, 24·90% I, 5·49% N; found: 47·09% C, 3·63% H, 24·80% I, 5·35% N. ^{1}H NMR: 1·82 s, 3 H (CH_3CONH); 2·23 s, 3 H (CH_3COO); 2·85 dd, 1 H (J_1 = 13·7, J_2 = 9·8) and 3·03 dd, 1 H (J_1 = 13·7, J_2 = 5, CH_2); 3·37 bs, 1 H (CHCONH); 4·59 m, 1 H (CH_2CH); 7·03 d, 2 H (H-3′, H-5′, J(3′, 2′) = J(5′, 6′) = = 8·7); 7·31 d, 2 H (H-2′, H-6′, J(2′, 3′) = J(6′, 5′) = 8·7; 7·65 dd, 1 H (H-5, J(5, 6) = 8·2; J(5, 3) = 1·8); 7·78 d, 1 H (H-6, J(6, 5) = 8·2); 8·33 d, 1 H (H-3, J(3, 5) = 1·8); 8·36 d, 1 H J = 7·9 ($NHCOCH_3$); 10·41 s, 1 H (COOH). ^{13}C NMR: 21·1 q; 22·6 q, 36·9 t; 55·4 d; 95·4 s; 118·4 d; 121·6 d; 121·6 d; 130·3 d; 130·3 d; 130·0 s; 130·9 d; 131·6 d; 135·2 s; 142·2 s; 149·3 s; 167·1 s; 169·4 s; 169·8 s; 171·2 s.

4-(N-Acetyl-L-tyrosyl)amino-2-iodobenzoic Acid (*XIII*)

A solution of diacetyl derivative *XII* (1·6 g) in 1M-NaOH (15 ml) was stirred at room temperature for 1·5 h. After acidification with 10% hydrochloric acid to pH 1, the separated precipitate was filtered and washed with water. The crude product was extracted with boiling light petroleum (10 ml) and then with ether (10 ml). The residue was repeatedly crystallized from aqueous methanol (charcoal). The obtained acid *XIII* (0·3 g; 20%) melted at 123—126°C (tetrahydrofuran–light petroleum); $[\alpha]_D$ +53° (*c* 0·45). ^{1}H NMR: 1·80 s, 3 H (CH_3CO); 2·72 dd, 1 H (J_1 = 13·9, J_2 = 9·4) and 2·89 dd, 1 H (J_1 = 13·9, J_2 = 5·8, CH_2); 3·32 bs, 1 H (CHCONH); 4·50 m, 1 H (CH_2CH); 6·63 d, 2 H (H-3′, H-5′, J(3′, 2′) = J(5′, 6′) = 8·5); 7·05 d, 2 H (H-2′, H-6′, J(2′, 3′) = = J(6′, 5′ = 8·5); 7·64 dd, 1 H (H-5, J(5, 6) = 8·6, J(5, 3) = 2·6); 7·76 d, 1 H (H-6, J(6, 5) = = 8·6); 8·24 d, 1 H ($NHCOCH_3$, J = 7·7); 8·31 d, 1 H (H-3, J(3, 5) = 2·6); 9·17 bs, 1 H (OH); 10·32 s, 1 H (COOH). ^{13}C NMR: 22·5 q; 36·9 t; 55·7 d; 95·3 s; 115·1 d; 115·1 d; 118·3 d; 127·7 s; 129·8 s; 130·2 d; 130·2 d; 130·8 d; 131·5 d; 142·2 s; 156·0 s; 167·1 s; 169·5 s; 171·4 s. For $C_{18}H_{17}$·.IN_2O_5 (467·9) calculated: 46·17% C, 3·67% H, 27·10% I, 5·98% N; found: 46·23% C, 3·62% H, 26·50% I, 5·91% N.

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