Selenium-Containing Pancreatic Imaging Agents. Synthesis of β-2- and β-3-Selenienylalanine (1)

T. Frejd, M. A. Davis**, S. Gronowitz* and T. Sadeh****

*Division of Organic Chemistry 1, Chemical Center, University of Lund, P. O. Box 740, S-220 07 Lund 7, Sweden; **Department of Radiology, Harvard Medical School, Boston, Mass. 02115, U.S.A.; *** Radiopharmaceuticals Unit, Soreq Nuclear Research Centre, Yavne, Israel

Received January 9, 1980

Selenophene-alanines, labelled with γ-emitting selenium-75, are potential diagnostic agents for disorders of the pancreas. Two of the positional isomers of selenienyl-alanine were synthesized, namely 2-selenienylalanine [2-amino-3-(2-selenienyl)propanoic acid] (88% yield) and 3-selenienylalanine [2-amino-3-(3-selenienyl)propanoic acid] (65% yield). Both amino acids were prepared from the known chloromethyl selenophenes (2- and 3-) by reaction with diethyl acetamidomalonate and subsequent hydrolysis. The method employed was found to be more productive than others which were tried.

J. Heterocyclic Chem., **17**, 759 (1980).

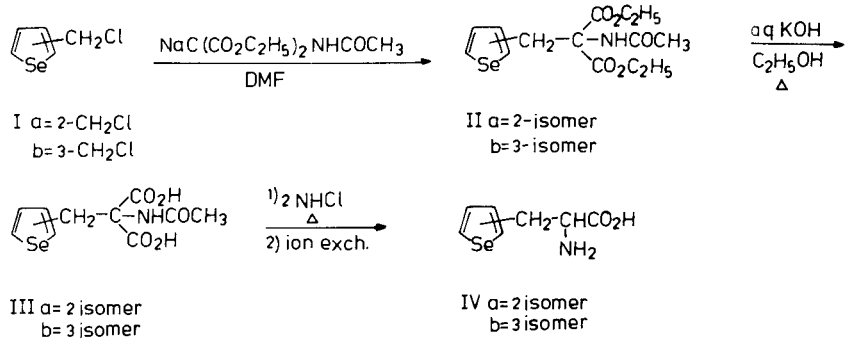
Certain amino acids labelled with radioactive isotopes have been shown to localize in the pancreas (2). Of these, ^{75}Se-selenomethionine is currently used as a pancreatic imaging agent for external visualization by γ-ray scintigraphy. However, this agent exhibits a small pancreas-to-liver concentration ratio (3a,b). Since the aromatic amino acids phenylalanine, β-2-thienylalanine and tryptophan possess superior pancreas-to-liver ratios (3a-c) than ^{75}Se-selenomethionine (3a,b), we have synthesized analogues of the former, namely β-2- and β-3-selenienylalanine. Their pancreas-to-liver ratios in rats, as determined by atomic absorption spectrometry, have appeared elsewhere and seem to be twice as good as that for ^{75}Se- L-selenomethionine (1).

The method used for the syntheses of β-2- and β-3-selenienylalanine (IVa and IVb, respectively, in Scheme 1) was essentially adapted from that used by Dittmer, *et al.* (4), for the preparation of β-2-thienylalanine. The advantage of the present method is that it is equally suitable for the preparation of gram quantities of both the β-2 and β-3 isomers of selenienylalanine, whereas the method described recently gives access only to the 2-isomer in low yield (5).

The chloromethyl derivatives Ia (6) and Ib (7), together with diethylacetamido malonate and sodium hydride in dimethyl formamide (DMF), gave IIa and IIb, respectively (see Scheme 1).

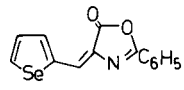
Due to the acid sensitivity of the selenophene ring (8), the hydrolysis of IIa with hydrogen bromide resulted in the formation of substantial amounts of elemental selenium. However, some amino acid was also formed, as demonstrated by a positive ninhydrin test. The destruction of the selenophene ring was circumvented by performing the hydrolyses of IIa and IIb in two stages: a basic hydrolysis of the ester functions gave IIIa and IIIb, which were further hydrolysed with 2*N* hydrochloric acid to give the hydrochlorides of amino acids IVa and IVb. These products were obtained pure by ion exchange chromatography on Amberlite CG 4B.

We also tried several other routes to IVa, namely the phosphorus-hydriodic acid reductive cleavage of azlactone V (9), reduction of the oxime VI (10) with sodium amalgam, rhenium heptasulfide catalyst and hydrogen (11), and reduction of thioketo acid VII (10) with sodium borohydride in concentrated aqueous ammonia. The azlactone V was, however, completely destroyed by the

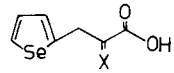


Scheme 1

0022-152X/80/040759-03$02.25



V



VI X=N-OH

VII X=S

vigorous conditions of the phosphorus-hydriodic acid reduction; the reduction of VI with sodium amalgam was incomplete and the attempted catalytic rhenium heptasulfide reduction of VI failed to proceed. The crude solutions, obtained after the sodium borohydride reduction of the thioketo acid VII and of the oxime VI showed positive ninhydrin reactions. The purification of the selenienyl alanine in these cases was complicated by the presence of large amounts of inorganic salts from the neutralization of the basic solutions with hydrochloric acid and was not attempted. It was not possible to precipitate VIa or VIb from solutions whose *p*H was adjusted to *p*H 5.5 by adding absolute ethanol, as was the case with β-2-thienyl alanine (4). β-2-Selenienyl alanine was found to be sparingly soluble in absolute ethanol, but the addition of water and heating increased its solubility considerably. Once in solution, it was difficult to recover the material even after refrigeration for several days at 5°. Recovery was less than 20%.

EXPERIMENTAL

Ethyl 2-Acetamido-2-carbethoxy-3-(2-selenienyl)propionate [Diethyl Acetamido-(2-selenienyl)malonate] (IIa).

Diethyl acetamidomalonate (20.6 g., 0.095 mole) was added in small portions to a mixture of 4.1 g. (~0.095 mole) of sodium hydride (55% in mineral oil) and 100 ml. of dry DMF. The mixture was then stirred for 1.5 hours. 2-Chloromethyl selenophene (17.0 g., 0.0947 mole; Ia) (6) was then added over 20 minutes with ice-cooling and the mixture was stirred for another hour. Most of the DMF was evaporated at reduced pressure. Water (300 ml.) was added to the residue, and the organic material was extracted with methylene chloride and dried (magnesium sulfate). Evaporation of the solvent gave an oil that crystallized after a while (26.7 g. crude yield). Recrystallization from ethanol-water gave 15.0 g. (44%) of IIa, m.p. 117-118°. The mother liquor was evaporated to half of its original volume but yielded no more crystals. Instead, a dark brown oil separated that did not crystallize and was not identified; nmr (deuteriochloroform): 1.30 (t, 6H, $-CH_3$), 2.05 (s, 3H, $-COCH_3$), 3.95 (s, 2H, 2-CH_2-), 4.25 (q, 4H, -O-CH_2-), 6.77 (b, 1H, > NH), 6.93 (m, 1H, H-3), 7.13 (dd, 1H, H-4), 7.85 (dd, 1H, H-5), $J_{4,5}$ = 5.4 Hz, $J_{3,5}$ = 1.0 Hz, $J_{3,4}$ = 3.6 Hz

Anal. Calcd. for $C_{14}H_{19}NO_5Se$ (360.27): C, 46.67; H, 5.32. Found: C, 46.89; H, 4.96.

Diethyl Acetamido-(3-selenienyl)malonate (IIb).

Using the procedure described above, IIb was prepared from 26.1 g. (0.145 mole) of Ib (6b), 6.5 g. (~0.15 mole) of sodium hydride (55% in mineral oil) and 32.4 g. (0.149 mole) of diethyl acetamidomalonate in 100 ml. of DMF; yield 30.6 g. (59%) of recrystallized IIb, m.p. 89.5-90.5° (from ethanol-water). Also in this case an unidentified oil was obtained from the mother liquor; nmr (deuteriochloroform): 1.28 (t, 6H, $-CH_3$), 2.04 (s, 3H, $-COCH_3$), 3.70 (s, 2H, $-CH_2$-), 4.28 (q, 4H, $-CH_2$-), 6.70 (bs, 1H, > NH), 7.10 (q, 1H, 4-H), 7.64 (bq, 1H, 2-H), 7.95 (q, 1H, 5-H), $J_{4,5}$ = 5.5 Hz, $J_{2,5}$ = 2.2 Hz, $J_{2,4}$ = 1.6 Hz, $J_{-CH_2-CH_3}$ = 7 Hz.

Anal. Calcd. for $C_{14}H_{19}NO_5Se$ (360.27): C, 46.68; H, 5.32; Se, 21.92. Found: C, 46.60; H, 5.30; Se, 21.97.

Acetamido-(2-selenienyl)malonic Acid (IIIa).

A solution of 15.1 g. (0.0419 mole) of IIa, 11.1 g. (0.109 mole) of potassium hydroxide, 100 ml. of water and 50 ml. of ethanol was refluxed for 12 hours and then evaporated to dryness. Another 100 ml. of water, together with 11 g. of potassium hydroxide were added. This solution was again refluxed for 3 hours, Norit was added and the mixture was cooled and filtered. The water phase was extracted with ether and acidified, yielding a crop of crystals (7.7 g.), which was filtered off. The filtrate was then extracted with 3 × 50 ml. of ether, which on evaporation deposited additional crystals (1.1 g.), total yield 8.8 g. (69%), m.p. ~125° (with decomposition and evolution of carbon dioxide). The compound is soluble in acetone and dimethyl sulfoxide but insoluble in chloroform; nmr (DMSO-d_6): 1.97 (s, 3H, $-CH_3$), 3.75 (bs, 2H, $-CH_2$-), 6.93 (m, 1H, H-3), 7.14 (dd, 1H, H-4), 7.79 (bs, 1H, > N-H), 7.97 (dd, 1H, H-5), $J_{3,4}$ = 3.6 Hz, $J_{3,5}$ = 1.2 Hz, $J_{4,5}$ = 5.4 Hz; ir (potassium bromide): C=O 1745 and 1715 cm^{-1}.

Anal. Calcd. for $C_{10}H_{11}NO_5Se$ (304.16): C, 39.49; H, 3.65; Se, 25.96. Found: C, 39.53; H, 3.67; Se, 26.00.

Acetamido-(3-selenienyl)malonic Acid (IIIb).

A mixture of 27.3 g. (0.0758 mole) of IIb, 20.0 g. (0.356 mole) of potassium hydroxide, 150 ml. of water and enough ethanol to make a homogeneous solution was stirred at room temperature for 24 hours and then refluxed for 3 hours. The mixture was cooled, treated with Norit, heated to boiling and filtered. After evaporation of most of the ethanol, the cold water phase was extracted with 50 ml. of ether and acidified. The water phase was placed in the refrigerator overnight for crystallization. A crop (17.7 g., 77%) of the title compound was obtained by filtration. The crystals lose carbon dioxide upon heating to 100-110°, resulting in the formation of *N*-acetyl-(3-selenienyl)alanine. Because of this ready loss of carbon dioxide, the elemental analysis was performed on the latter compound, m.p. 143-145°; nmr (DMSO-d_6): 1.82 (s, 3H, $-CH_3$), 2.84-3.14 (m, 2H, $-CH_2$-), 4.20-4.68 (6 lines, 1H, -CH-), 7.27 (dd, 1H, H-4), 7.81 (dd, 1H, H-4), 8.06 (dd, H-5), 8.14 (d, > N-H) (integral at 8.06 and 8.14 = 2H), $J_{2,5}$ = 2.4 Hz, $J_{2,4}$ = 1.2 Hz, $J_{4,5}$ = 5.2 Hz, $J_{> NH,CH}$ = 7.6 Hz; ir (potassium bromide): > C=O 1605 and 1690 cm^{-1}, > N-H, 3330 cm^{-1}.

Anal. Calcd. for $C_9H_{11}NO_3Se$ (260.15): C, 41.55; H, 4.26; Se, 30.32. Found: C, 41.51; H, 4.23; Se, 30.27.

2-Selenienyl Alanine (IVa).

A solution of 1.0 g. (3.3 mmole) of IIIa in 25 ml. of 2*N* hydrochloric acid was refluxed for 17 hours, after which the solution was evaporated to dryness. The residue of 2-selenienyl alanine hydrochloride weighed 0.74 g. and was dissolved in 50 ml. of deionized water and filtered through an anion exchange resin (5 ml. of the acetate form of Amberlite CG 4B). The resin was then washed with 4 × 10 ml. of deionized water. The filtrate, on evaporating to dryness, deposited pure IVa, yield 0.63 g. (88%), m.p. (with decomposition) ~210°; ir (potassium bromide): 1625 (NH_3^+), 1590 ($COO^\ominus$) cm^{-1}; nmr (trifluoroacetic acid): 3.84 (m, 2H, CH_2), 4.69 (q, 1H, CH), 7.30 (bm, H-3, H-4 and acidic protons), 8.08 (q, 1H, H-5, assigned to H-5 due to exchange with deuteriosulfuric acid).

Anal. Calcd. for $C_7H_9NO_2Se$ (218.11): C, 38.55; H, 4.16; Se, 36.20. Found: C, 38.60; H, 4.13; Se, 36.14.

3-Selenienyl Alanine (IVb).

This compound was prepared by the same procedure as descibed for IVa. From 6.1 g. (0.020 mole) of crude IVb in 150 ml. of 2 *N* hydrochloric acid a yield of 4.64 g. of crude amino acid hydrochloride was obtained, which was dissolved in 50 ml. of deionized water and placed on 30 ml. of Amberlite CG 4B (acetate form). The amino acid was eluted with 5 × 25 ml. of deionized water. After evaporating to dryness and drying the residue in a desiccator over phosphorus pentoxide, 2.85 g. (65%) of the pure IVb was obtained, m.p. ~260° dec; ir (potassium bromide): 1630 (NH_3^+), 1580 ($COO^\ominus$) cm^{-1}; nmr (trifluoroacetic acid): 3.55 (m, 2H,

-CH_2-), 4.62 (m, 1H, CH), 7.28 (m, aromatic and acidic protons), 8.09 (m, 2H, aromatic).

Anal. Calcd. for $C_7H_9NO_2Se$ (218.11): C, 38.55; H, 4.16; Se, 36.20. Found: C, 38.48; H, 4.15; Se, 36.29.

Acknowledgement.

Grants from the Swedish Natural Science Research Council to S. G. and from the Royal Physiographic Society in Lund to T. F. are gratefully acknowledged.

REFERENCES AND NOTES

(1) Presented in part at the 24th Annual Meeting of the Society of Nuclear Medicine, Chicago 1977.

(2) E. Hansson, *Acta Physiol Scand. Suppl.*, 161 (1969).

(3a) M. A. Davis, R. W. Giese, H. T. Norton and T. Sadeh, *Chem. Scr.*, **8a**, 108 (1975); (b) D. M. Taylor and M. F. Cottrall, "Radiopharmaceutical and Labelled Compounds Proc. Symp", Vol. 1, IAEA, Vienna, Austria 1973, p. 433; (c) H. Busch, J. R. Davis, G. R. Honig, D. C. Anderson, P. V. Nair, and W. L. Nyhan, *Cancer Res.*, **19**, 1030 (1959).

(4) K. Dittmer, W. Herz and J. S. Chambers, *J. Biol. Chem.*, **166**, 541 (1946-47).

(5) P. M. Jacobs and M. A. Davis, *J. Org. Chem.*, **44**, 178 (1979). By a misunderstanding, this paper was published before the present paper was submitted for publication.

(6) Yu. K. Yur'ev and N. N. Mezentsova, *J. Gen. Chem. USSR*, **27**, 201 (1957) (English translation).

(7) Yu. K. Yur'ev, N. N. Magdesieva and A. T. Monakhova, *Khim. Geterotsikl. Soedin.*, **4**, 474 (1968) (English translation).

(8) S. Gronowitz and T. Frejd, *Acta Chem. Scand.*, **26**, 2279 (1972).

(9) Yu. K. Yur'ev, N. N. Mezentsova and T. A. Balashova, *J. Gen. Chem. USSR*, **27**, 2594 (1952) (English translation).

(10) Yu. K. Yur'ev and N. N. Mezentsova, *ibid.*, **28**, 3071 (1958).

(11) M. A. Ryashentseva, Kh. M. Minachev, O. A. Kalinoskii and Ya. L. Goldfarb, *Zh. Org. Khim.*, **1**, 1104 (1965) (English translation).