# SYNTHESIS OF $^{75}$Se-2-PHENYL-1,2-BENZISOSELENAZOL-3(2H)-ONE (PZ 51; EBSELEN*). A NOVEL BIOLOGICALLY ACTIVE ORGANO-SELENIUM COMPOUND.

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## SUMMARY

The preparation of $^{75}$Se-ebselen ($^{75}$Se-PZ 51) in a high radiochemical yield (∿ 40 %) and with a specific activity of 240 mCi/mM (8.9 GBq/mM) is described. It entails a very simple, fast and one-pot procedure starting from elemental $^{75}$Se-selenium.

$^{75}$Se-diselenosalicylic acid 4 is initially prepared as the key intermediate which is transformed into a corresponding dichloride 5-before reacting with aniline to yield the desired $^{75}$Se-ebselen.
Identity and purity of the labelled compound were controlled by comparison in TLC, HPLC and MS with an authentic sample (1-2).

Keywords : $^{75}$Se-radiosynthetics, 2-phenyl-1,2-benzisoselenazol-3(2H)-one, PZ 51, ebselen.

## INTRODUCTION

Ebselen* 6 is a new synthetic organo-selenium drug with extremely low toxicity ($LD_{50}$ in rats > 6180 mg per os) and showing in vivo antiinflammatory properties (3).

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Recent investigations have shown that ebselen exhibits glutathione peroxidase-like and antioxidant activity, offering a novel approach to anti-inflammatory therapy (4, 5, 6, 7).

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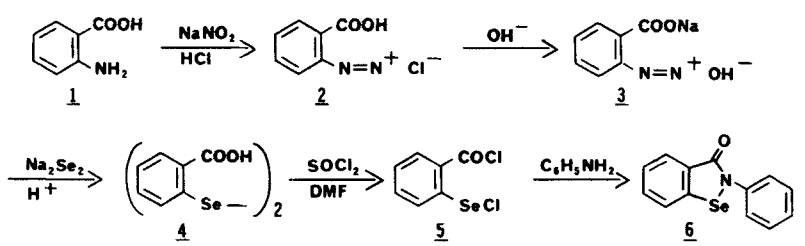
In order to investigate the metabolism and the pharmacokinetics of ebselen in animal experiments, labelled compound was required. A very important investigation was to check the possible availability of the selenium moiety for the in-vivo synthesis of the naturally occurring glutathione peroxidase. Therefore a $^{75}Se$-label was the obvious choice.

$^{75}Se$-ebselen allowed Wendel et al. (5) to show that the selenium atom is not incorporated into endogeneous glutathione peroxidase. The metabolic and pharmacokinetic investigations will be published elsewhere.

## RESULTS AND DISCUSSION

Diselenosalicylic acid 4 was first prepared by the reaction of diazonium salt 2 with sodium diselenide generated by reduction of elemental selenium with rongalite (1). The isolated yields are excellent on a molar-scale. When it was first tried to transpose this reaction to a microscale, yields were very disappointing. Yields of $^{75}Se$-diselenosalicylic acid could be improved drastically by reacting 2 with sodium diselenide arising from the reduction of elemental selenium with sodium borohydride (8).
The chemical pathway is detailed in the following scheme:




A minimal amount of 500 mg (1.25 mmol) of diselenosalicylic acid is required for achieving substantial chemical and radiochemical yields in the thionyl chloride reaction (4 - 5). With smaller amounts of compound 4, even

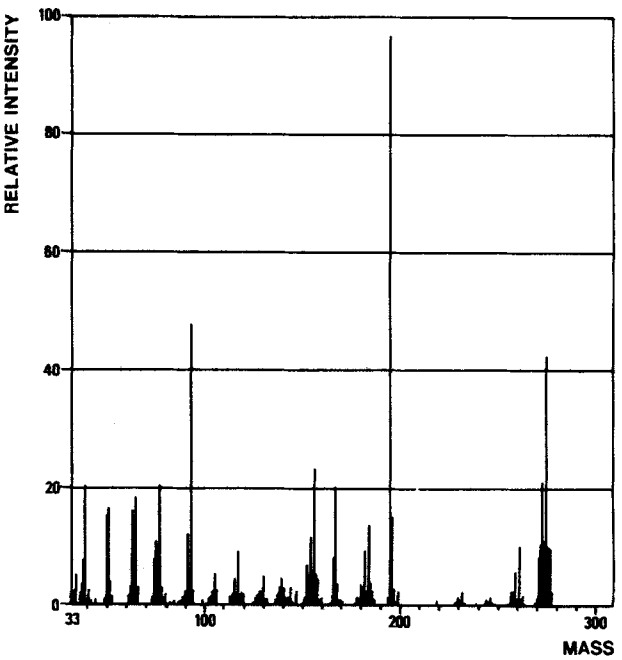



Figure 1.
Low resolution Mass Spectrum 70 eV of PZ51.

| Mass | 195 | 93 | 275 | 156 | 273 | 167 | 39 | 77 | 65 | 51 |
|---|---|---|---|---|---|---|---|---|---|---|
| Intensity | 10000 | 4930 | 4365 | 2456 | 2202 | 2122 | 2120 | 2104 | 1903 | 1718 |

under the most drastic conditions of dryness, the chemical yield decreases dramatically. Conditions for the microscale radioactive synthesis (1 µmol of 4) are now under study.

Two methods have been investigated to reduce $Se^{o}$ into $Se^{-1}$

. firstly, a method using only sodium borohydride as reducing agent;
. secondly, a reduction of elemental selenium by a combined action of sodium borohydride and hydrazine hydrate.

In the first cas, the reduction does not stop on the $Se^{-1}$ oxidation state and the formation of $Se^{-2}$ cannot totally be avoided. This latter is responsible for

the formation of the main selenium consuming chemical impurity, the monoselenosalicylic acid. Experience showed that the color change in the reaction mixture was the only factor related to the end of the reaction : $Se^0$ (clear suspension in NaOH 0.1 M) → $Se^{-1}$ (red) → $Se^{-2}$ (colorless). It is therefore recommended in the reaction step 3 - 4 to add the neutralized diazonium salt solution before total completion of the selenium reduction.

The second method has the advantage that the monoselenosalicylic acid cannot be formed as hydrazine hydrate reduces $Se^0$ only into $Se^{-1}$ oxidation state (9).

Chemical identity was established for the final ebselen compound 6 by comparing its retention parameters, both in TLC and HPLC and mass spectrum (fig. 1) with those of an authentic standard. TLC results indicated the presence of single radioactive compound (> 99 %) which presents the same value of Rf :0.65 as the non-radioactive authentic sample on silica gel plates and using a benzene-ethyl acetate (50:50) mixture as the solvent.

The radiochemical purity of ebselen 6 was checked also by both TLC and HPLC. HPLC confirmed the TLC results. No contaminant peaks were found. Radiochemical yield (40 %) and specific activity were easily assessed from the Ge-Li $\gamma$ spectrometry measurements of the radio HPLC peaks.

For our animal studies, a specific activity of 5 mCi/mmole is required. Higher specific activities are necessary for the study of the loading effect on the in vivo biodistribution profile. The compound Ebselen 6 was stable at room temperature.

## EXPERIMENTAL

. Materials and Analytical Procedures :

Organic solvents and reagents were analytical grade (p.a.) from Merck and Aldrich Chemical Co, and were used without further purification. High purity (99.9 %) powdered black selenium (100 - 200 mesh) was obtained from Societé Générale des Minerais Hoboken Belgium. Radioactivation of 10 mg sample

placed in a sealed quartz ampoule was performed in the thermal flux ($510^{14}$ n /cm$^2$. sec) of the BR2 (Belgian reactor n°2) for a period of 18 days.

The specific activity obtained under these conditions is about 0.25 Ci/mM ( ∿ 9 GBq/mM). By using an enriched (40 % $^{74}$Se) target a specific activity of 10 Ci/mM (370 GBq/mM) might be easily obtained under the same conditions.

The organic substrates were all purchased from Aldrich except sodium borohydride and hydrazine hydrate which were obtained from Fluka. Precoated TLC plates, silicagel - 25 UV 254 were obtained from Macherey-Nagel Co (Düren - FRG) and chromatographed with the benzene-ethyl acetate (50:50 v/v) system.

Radio HPLC was carried out using a Model 6000 A from Waters equipped with a 254 nm UV detector and coupled to a collimated Na I (2" x 2") scintillator associated to a monochannel γ spectrometer from Eberline. A 100 mm μ -Bondapack C-18 column from Waters was used in all separations with water/methanol mixtures (65:35; v/v) as the mobile phase. HPLC was use as a preparative technique as well.

Mass spectrometry was carried out using the direct injection mode at 70 eV with a LKB model 2091 mass spectrometer equipment (Bromma, Sweden).
All the radioactivity measurements were performed by γ spectrometry.
A 2.5 KeV resolution, 16 cm$^3$ volume Ge-Li detector was used.

. Reactions :

Synthesis of anthranilic diazonium salt 3.

Sodium nitrite (0.6 mmol) in 1 ml water was added dropwise to a stirred solution of anthranilic acid (0.6 mmol) in 2 ml HCl 1N. The reaction temperature was kept below 10°C by external cooling with an ice-bath.
The final mixture was made alkaline by addition of sodium hydroxide solution (2 N).

Synthesis of $^{75}$Se-diselenosalicylic acid $^{75}$Se 4.

Into a 25 ml three-necked flask fitted with a reflux condenser, a 10 ml addition funnel and an argon inlet, were placed 50 mg (0.64 mmol - 150 mCi) of powdered black $^{75}$Se-selenium suspended in 3 ml of sodium hydroxide solution 1 N. The flask was cooled to keep temperature between 0°C and 5°C with an external ice bath and flushed with a continuous flow of argon. 1 ml of a sodium borohydride solution (0.66 M in NaOH 1 N) freshly prepared was added to reduce the elemental selenium to the -1 oxidation state. The reduction of $Se^0$ can also be started with the minimum of sodium borohydride and completed with a equimolar quantity of hydrazine hydrate.

The diazonium salt solution was then progressively added under vigorous stirring. The stirring of the resulting brown-yellow solution was continued under an argon stream for 20 - 30 minutes. The solution was then transferred to a 100 ml centrifuge tube and by addition of concentrated HCl the diselenosalicylic acid was precipitated. The latter was centrifuged, the supernatant discarded and the yellow precipitate washed with water and finally dried overnight at 60°C. The total weight of diselenosalicylic acid was brought to 500 mg by addition of pure non-labelled compound.

Synthesis of $^{75}$Se-2-Phenyl-1,2-benzisoselenazol-3(2H) one ($^{75}$Se-ebselen).

Thionyl chloride (5 ml) and dimethylformamide (50 µl) were added to 0.5g (1.25 mmol) of dried diselenosalicylic acid. The resulting suspension was refluxed under stirring for 1 h. The mixture was evaporated to dryness under reduced pressure and the residue dissolved in $CCl_4$ (20 ml). 1 ml of aniline in 10 ml of $CCl_4$ was added dropwise with stirring. An ebselen precipitate was formed immediately. The solid obtained was filtered or centrifuged and washed with HCl 0.1 N, then water, to provide the desired compound 6 after being dried overnight at room temperature.

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REFERENCES

(1) R. LESSER and R. WEISS.
Ber. 57 : 1077 (1924).

(2) R. WEBER and M. RENSON.
Bull. Soc. Chim. France 7/8 : 1124 (1976)

(3) a. Euro. PAT 44971 A. NATTERMANN & Cie GmbH
Ger. Offen 3027073 A. Nattermann & Cie GmbH.

b. N. DEREU and E. GRAF.
Drugs of the Future 9 : 741 (1984).

c. M.J. PARNHAM, S. LEYCK, N. DEREU, J. WINKELMANN and E. GRAF.
Adv. Inflam. Res. 1985, in press.

(4) M.J. PARNHAM and S. KINDT.
Biochem. Pharmac. 33 : 3247 (1984).

(5) A. WENDEL, M. FAUSEL, H. SAFAYHI and R. OTTER.
Biochem. Pharmac. 33 : 3241 (1984).

(6) A. MÜLLER, H. GABRIEL and H. SIES.
Biochem. Pharmacol., 1985, in press.

(7) A. MÜLLER, E. CADENAS, P. GRAF, H. SIES.
Biochem., Pharmac. 33 : 3235 (1984).

(8) D.L. KLAYMANN and T.S. GRIFFIN.
J. Am. Chem. Soc. 95 : 197 (1973).

(9) L. SYPER, J. MLOCHOWSKI.
Synthesis 439 (1984).