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SYNTHESIS AND STABILITY OF SULFUR-35 LABELLED 4-ETHYLBICYCLOTHIOPHOSPHATE

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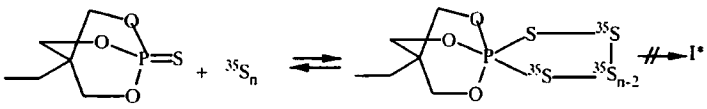
Different synthetic routes to the preparation of 4-ethyl-2,6,7-trioxa-1-phosphabicyclo[2,2,2]octane-1-thioxide labelled with sulfur-35 have been investigated since it is of considerable interest as radioligand for neurochemistry. The thiophosphate was successfully synthesized by sulfuration of the corresponding bicyclophosphite by elemental sulfur-35, however the radiochemical purity of the material obtained in this way proves to be low. Convenient methods for synthesis of the thioxide from [^{35}S] thiourea, based on the results of the autoradiolysis of the starting [^{35}S] thiourea, has been discovered giving rise to thioxide with high radiochemical purity. The autoradiolytic stability of the labelled thiophosphate has been studied.

4-Alkyl-2,6,7-trioxa-1-phosphabicyclo[2,2,2]octane-1-thioxides (bicyclothiophosphates) [1] are selectively active neurotropic materials able to suppress the neuronal effects of γ-aminobutyric acid. Several bicyclothiophosphates, being selective blockers of Cl^- canal, have extremely high toxicity [1, 2] and physiological activity [1, 3] and compounds of this class labelled by radioisotopes are used at this time as radioligands for neurochemistry and neuropharmacology [1, 4, 5].

The aim of our work was a search for the optimal methods for synthesizing sulfur-35 labelled 4-ethylbicyclothiophosphate I^*.

As one possible method for the synthesis of thiophosphate I^* we examined isotopic exchange in the system 4-ethylbicyclothiophosphate I and elemental sulfur-35 which has not been studied heretofore. We started from the proposal that (in contrast to noncyclic derivatives of thiophosphoric acid [6] which do not exchange with elemental sulfur-35) compound I, as a result of the better steric availability of the phosphorus atom in the structure and the ability to form a spiro compound [1], is able also to form the intermediates II and hence to take part in isotopic exchange.



I II

However, we have found that this process does not occur in the case of compound I. Analysis of the reaction mixtures was carried out by thin-layer radiochromatography. Reactions were carried out in toluene, in a mixture of toluene and amyl alcohol (1:1) and without a solvent at different temperatures (60-120°C). When carrying out the reaction without solvent, even at temperatures less than 100°C, a marked breakdown of compound was observed with insignificant incorporation of the radioactive label.

There are known two basically different methods for synthesizing bicyclothiophosphates. The first is based on the phosphorylation of the corresponding triols with thiophosphoryl chloride which, however, does not give a

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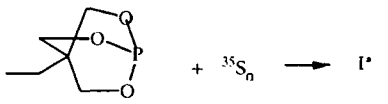
high yield. Additionally, in this case the introduction of the radioactive label does not occur at the last stage of the preparation of bicyclothiophosphate, hence the method is not generally applicable for radiochemical synthesis. The second method consists of sulfuration of the corresponding bicyclophosphite by an appropriate sulfur-containing reagent. Elemental sulfur [7, 8] and mercaptans [1] have been used as the latter. In our work we have shown that this route is applicable to the synthesis of compound I* labelled with sulfur-35, but the yield and radiochemical purity depend markedly on the reaction conditions.

The starting 4-ethylbicyclophosphite III was successfully thionated using elemental sulfur-35 reagent:



III

When carrying out the reaction without solvent (in molten reagents in an inert atmosphere) the yield of compound I* is not more than 15% but, when using toluene, *p*-xylene, or a mixture of toluene and amyl alcohol (1:1) as reaction medium, the yield is satisfactory (Table 1). Addition of sodium sulfide does not increase the yield. The thiophosphate I* (as all of the class of bicyclophosphates [1]) is an extremely labile structure which is shown by the significantly lower yield with a reaction longer than 6 h. We also found that the yield is lowered with increase of the specific radioactivity of elemental sulfur which may be a result of the high radiation sensitivity of compound I* and this is borne out by the significant autoradiolytic effects for this material on storage.

Despite the satisfactory yield of compound I*, its radiochemical purity proved to be low and removal of the radioactive impurities by usual methods (crystallization, extraction, etc.) was ineffective (the purity after several crystallizations and purification on an HPLC column was about 95%). Hence we studied the possible use of other sulfurating agents. It is known [1, 9] that phosphines and phosphites react with thiols and disulfides in the presence of initiators of radical processes to form the corresponding thioxides. The Lawesson reagent readily sulfurates compounds of trivalent phosphorus [10] and, under its influence, it is also possible to exchange the oxygen atom of

TABLE 1. Radiochemical Yield (%) of Thiophosphate I* upon Reaction of Phosphite III (C_0 = 1.5 mole/l) with Elemental Sulfur-35 (C_0 = 1.5 mole/l) in Different Conditions

Conditions	Time, h	Specific activity of sulfur-35, mCi/mmole	Radiochemical yield*, %
p-Xylene, 138°C	1	0.5	30
p-Xylene, 138°C	4	0.5	43
Toluene, 110°C	1	0.5	22
Toluene, 110°C	4	0.5	65
Toluene, 110°C	6	0.5	78
Toluene, 110°C	10	0.5	50
Toluene, 90°C	10	0.5	30
Toluene, 90°C	25	0.5	39
Toluene, 110°C	6	5.0	54
Toluene, 110°C	6	60	<30*[2]
Toluene–1-pentanol, 1:1, 110°C	6	0.5	71
Toluene–1-pentanol, 1:1, addition of Na_2S (0.1 mole/l), 110°C	6	0.5	70

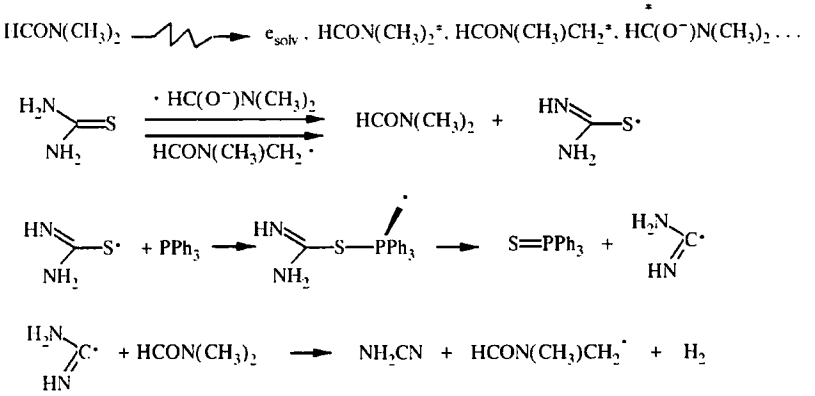
* Ratio of radioactivity of compound I* to radioactivity of all labelled materials in the reaction mixture.

*[2] Large number of side products observed.

the P=O group for sulfur in phosphine oxides [11]. However, in the radiochemical synthesis the indicated methods of sulfurating derivatives of trivalent phosphorus were little suited since they need a laborious and multistage preliminary synthesis of the labelled reagents.

In the present work we have studied the use of [^{35}S] thiourea as the source of sulfur-35 the sulfuration of phosphite III, since it is an available and convenient reagent. As a model reaction we studied the interaction of triphenylphosphine with thiourea under different conditions (in the presence of CCl_4 or inhibitors of radical processes, in toluene or methylene chloride, at room temperature, and upon refluxing for 2-4 h) and analyzed the reaction mixtures by ^{31}P NMR spectroscopy. Previously PPh_3 in the presence of CCl_4 has been used [12] for desulfurizing thioureas with the aim of producing carbodiimides, however the triphenylphosphine transformation products were not isolated in this reaction. In all cases, in our hands, triphenylphosphine thioxide was formed. Thus a marked amount of it was found on refluxing equimolar quantities of PPh_3, CCl_4, and thiourea in methylene chloride for 4 h, 40% of $S{=}PPh_3$ being produced. In the presence of azoisobutyronitrile (AIBN) the reaction is complicated by the direct interaction of thiourea with radical species, none the less $S{=}PPh_3$ was also detected in the reaction mixture (up to 10% according to NMR spectral data).

In the present work we have found for the first time that thiourea, labelled with sulfur-35, reacts with PPh_3 to give [^{35}S] $S{=}PPh_3$. The reaction takes place in a mixture of refluxing toluene and alcohol (1:1). Its rate is increased with an increase in the specific radioactivity of thiourea which points out a decisive role for autoradiolysis of the labelled thiourea in its reaction with PPh_3. Hence, for a specific activity of [^{35}S] thiourea of 10.0 mCi/mmole, the reaction reaches 50% conversion stage approximately three times faster than for using a reagent with specific activity of 0.5 mCi/mmole. It is likely that the reaction has a radical-chain character, and that its mechanism analogously to the radical reaction between PPh_3 and thiols [13] can be depicted by the following sequence of reactions including formation of thiiyl and phosphoranyl radicals. We carried out a radiation-chemical modelling of this unusual reaction and found that thiourea and triphenylphosphine also indeed react under the influence of γ-irradiation. Analysis of the reaction mixtures was carried out using ^{31}P NMR spectroscopy. The most rapid process takes place in DMF (concentration of each reagent about 1 mole/liter). In this way about 2% of thioxide are formed for 100 rad of external γ-irradiation. The conditions of the radiation-chemical experiment are similar to those given in [14].



Reaction of labelled thiourea with triethyl phosphite and bicyclophosphite III is also observed, however alkylation of thiourea proceeds parallel with it.

Although the yield of thioxide I* in the reaction of phosphite III with [^{35}S] thiourea *via* autoradiolysis is inferior to the yield of sulfuration of phosphite III by elemental [^{35}S], at the same time the radiochemical purity of the product in the first case occurs to be significantly higher (more than 98.5% after one crystallization). In addition, its separation and purification proved to be more convenient.

Using thin-layer radiochromatography we have studied the dependence of the radiochemical purity of thioxide I* on the conditions of storage and with different stabilizing additives (Table 2).

TABLE 2. Dependence of Radiochemical Purity of Thioxide I* (0.010 Ci/mmole) on Storage Conditions in Solutions with Radioactive Concentration of 0.5 mCi/ml

Solvent	Temperature, °C	Initial radiochemical purity, %	Radiochemical purity, %	
			after 1 month	after 3 months
Ethanol	+20	98	92	80
Ethanol	+5	98	90	80
Ethanol	-20	98	98	94
Without solvent	+5	98	96	90
5% ionol in ethanol	+5	98	98	95
Ethanol	+5	95	80	less than 50
Ethanol–*p*-xylene, 1:1	-20	95	85	70
Without solvent	+5	95	90	75
5% ionol (+ PPh_3) in ethanol	+5	95	90	85

For determining the radiochemical purity of thioxide I* we compared the radioactivity of the corresponding chromatographic zone with the activity of the whole chromatogram.

In our work we have found (Table 2) that the most efficient means of inhibiting the autoradiolysis of thioxide I* (besides its careful purification before the storage) over time is stabilization by addition of ionol.

With the aim of comparison of stability, we have synthesized compound I (using solid-phase heterogeneous isotopic exchange [15]) labelled with tritium with a specific activity of about 20 Ci/mmole. After chromatographic purification, its radiochemical purity was better than 98%. We have found that [^{3}H]-labelled thioxide I is extremely stable upon storage (in alcohol or in a mixture of alcohol and toluene at 5°C) for one year and only then, in terms of main radiochemical impurity, begins to form labile tritium (not more than 5-10% in a year).

EXPERIMENTAL

Monitoring of the radiochemical purity of thioxide I* and of the compounds in its synthesis was carried out using a radiochromatographic method. In order to do this, the sample was placed on a Silufol silica gel plate, the chromatograms developed in systems 1 to 4 (see below), and revealed using the Grot reagent or iodine vapor. After development and fixing the zones corresponding to the starting labelled materials and reaction products were cut out and placed in small bottles containing scintillation liquid. In all cases distinct separation of the starting material and reaction products was observed. For determination of the purity of thioxide I* the radioactivity of the corresponding chromatographic zone was compared with the activity of the entire chromatogram and the chromatographic analysis was carried out in all the systems 1 to 4. In several cases nonradioactive carriers (standard materials) were applied to the silica gel plates at the same time as the analyzed sample in order to separate and identify the reaction products.

System 1: butanol–acetone–formic acid, 1:0.8:1. System 2: benzene–methanol, 2:1. System 3: hexane–acetone, 3:1. System 4: water–DMF, 5:1.

Measurement of radioactivity was carried out in Mark II and SL-4200 liquid scintillation counters, using standard scintillation mixtures (e.g., 4 g of PPO and 0.2 g of POPOP in 1 liter of toluene). PMR and ^{31}P NMR spectra were recorded on a Bruker -200 spectrometer (working frequency 81 MHz for ^{31}P nuclei). TMS and H_3PO_4 were used as external standards.

4-Ethyl-2,6,7-trioxa-1-phosphabicyclo[2,2,2]octane (III) was prepared according to the method [7] starting from trimethylolpropane and triethyl phosphite. Yield 58%; mp 56°C. δ ^{31}P 93 ppm.

4-Ethyl-2,6,7-trioxa-1-phosphabicyclo[2,2,2]octane-1-thioxide (I). Solution of phosphite III (0.25 g, 1.5 mmol) and elemental sulfur (0.049 g, 1.5 mmol) was refluxed in toluene (5 ml) for 6 h. The precipitate was filtered off and washed with toluene and carbon disulfide. Yield 82%; mp 175-177°C. R_f 0.33 (system 2) and R_f 0.25 (system 4). δ ^{31}P 68 ppm.

Synthesis of triphenylphosphine sulfide was carried out by two methods. A. Starting from triphenyl phosphine and elemental sulfur by heating the mixture in toluene. Yield 81%; mp 158-159°C. δ ^{31}P 42.9 ppm. B. Starting from PPh_3, CCl_4, and thiourea by heating the mixture in the presence of triethylamine. Yield 40%; mp 162°C. δ ^{31}P 42.6 ppm.

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