Synthesis of Specific Labelled [Methyl-^{14}C]Sarin

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Summary

Synthesis of specific labelled [methyl-^{14}C]sarin.

The synthesis of labelled [methyl-^{14}C]sarin, [^{14}C]methylphosphonofluoridic acid 1-methylethyl ester, was accomplished by another approach as for nonlabelled sarin in a tele-conducted reaction vessel. The purity was estimated by IR, GC, and GC-MS and the stability in different media was examined.

Key words: Specific labelled sarin, tele-conducted reaction vessel.

INTRODUCTION

Sarin, methylphosphonofluoridic acid 1-methylethyl ester was first synthesized by Schrader (1) . Furtheron numerous syntheses of sarin, together with syntheses of similar organophosphorous compounds were published by Tammelin (2), Bryant et al. (3) and Franke (4). The first radioactive synthesis with ^{32}P-sarin was published by de Borst (5) . Our aim was to synthesize the best suited substrate for acetylcholinesterase, acetylcholine acetyl-hydrolase, EC 3.1.1.7 (ACHE), serving for structure evaluation of the active centre and for pharmacological studies with animal autoradiography.

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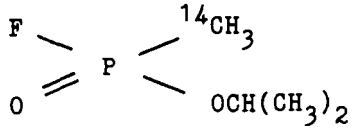
As sarin is highly toxic and sensitive against hydrolysis we only synthesized small amounts for immediate use.

Formula 1 [Methyl-^{14}C]sarin (2*)



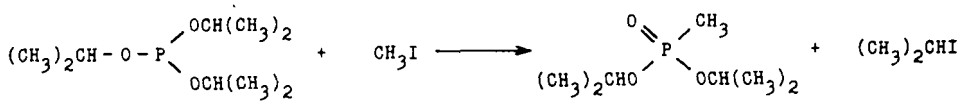
2*

The following considerations served as a basis for our reasoning to use [methyl-^{14}C]sarin.

1. Sarin is the best known inhibitor of AChE.
2. According to the long half-life but low radiotoxicity the ^{14}C-labelled compounds are suited for animal autoradiography.
3. Compared to the hydrolizable isopropyl group the rigid P-^{14}C-methyl group proved to be best suited for our purpose.
4. ^{32}P-labelled compounds are less suited because of their low half-life but higher radiotoxicity (6, 7, 8).

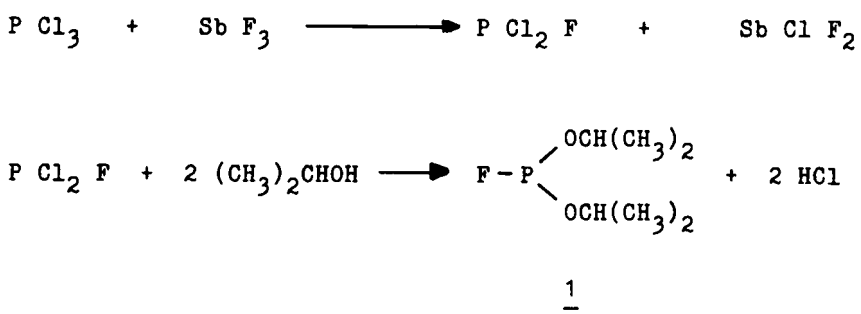
Childs and Williams (9), in analogy to the Arbusov reaction (10a and b, 11, 12) found - Scheme 1 -, that sarin (2) is formed by heating a mixture of methyliodide and phosphorofluoridous acid diisopropylester (1).

Scheme 1 Arbusov Reaction



Childs and Williams synthesized sarin (2) according to the following way (13 , 14 , 15). Scheme 2 .

Scheme 2 Childs and Williams Reaction



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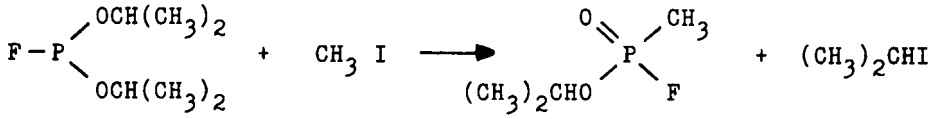
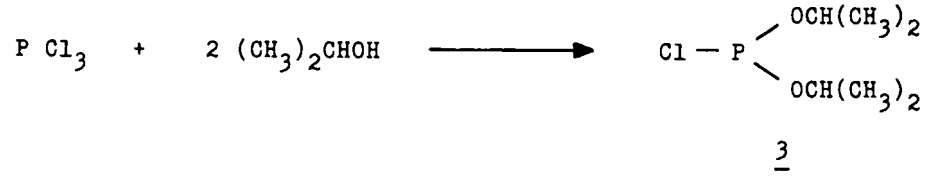


1 2

However,the simplified method according to Houben Weyl (10b), Kuhn and Olah (16), treating phosphorochloridous acid diisopropyl ester (3) with SbF_3 proved to be more suitable for our purpose (Scheme 3).

Scheme 3 Synthesis of [methyl-^{14}C]sarin (2*)



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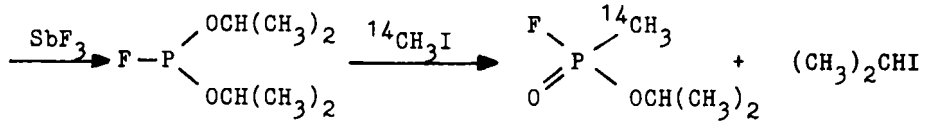
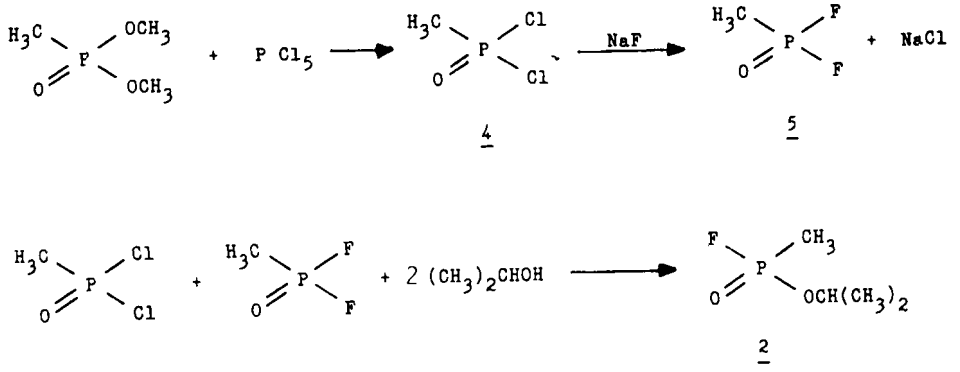


1 2*

The synthesis of non-labelled sarin was accomplished according to the following procedure (13), Scheme 4 .

Scheme 4 Synthesis of non-labelled sarin



In this way non-labelled sarin was synthesized with 80% yield and 99% purity (GC).

In order to avoid radioactive contamination, poisoning and access of humidity to the reaction mixture we used a microreaction vessel. The synthesis of [methyl-^{14}C]sarin yielded 84% with 85% purity (GC).

EXPERIMENTAL

With regard to the extreme toxicity of sarin to human beings, extreme care was taken at all following procedures.

Infrared analysis (IR): Beckmann Acculab 4. Solvent carbontetrachloride, "CCl_4 für die Spektroskopie, Merck".

- Phosphorofluoridous acid diisopropylester (1)

IR (CCl_4, cm^{-1}): 2980, 2940, 2900, 2880 C-H stretching. Doubletts 1465, 1450, and 1385, 1370 CH -deformation. 1355 CH-deformation. 1175, 1135 and 880 (isopropyl). 1100 und 1110 O-C stretching. 995 and 950 (P-O). 860 P-F stretching.

- Sarin (2)

IR (CCl_4): 3010, 2970 and 2900 C-H of isopropyl. 1465, 1450, 1420, 1390 and 1375 deformation CH and isopropyl. 1315 P-CH.

1280 P=O stretching. 1175, 1140 and 895 P-O-CH-$(CH_3)_2$. 1120 (shoulder) and 1100 O-C stretching P-O-CH. 920 and 895 deformation P-O-CH. 835 P-F stretching.

- [Methyl-^{14}C]sarin (2*)

The IR-spectra of non labelled and labelled sarin are corresponding with those of Lorquet and Vassart (17). Two shoulders at 1260 and 980 may be attributed to minor impurities. The purity of non labelled and labelled sarin was detected by gas chromatography (GC) and gas chromatography/mass spectrometry (GCMS).

GC: Carlo Erba Fractovap Mod. G1. Column 1.5 m, i.d. 2.5 mm 4% Ucon HB 5100, Chromosorb G, AW, DMCS, 80 mesh. Gasflow nitrogen 0.8 atm (30 ml/min.). Detector: FID, hydrogen 0.8 atm, air 1.2 atm. Temperature: Column 100°C, injektor 230°C.

The main peak with a retention time of 1.5 min. was identified to be [methyl-^{14}C]sarin (A). The peaks (B), (C), and (D) were identified to be unessential by-products. Peak (D) was estimated to be [methyl-^{14}C]phosphonic acid diisopropylester, peaks (B) and (C) could be identified as phosphorofluoridic acid diisopropylester (B), and phosphonic acid diisopropylester (C), respectively (Table 1). According to a 1,2,3,4-tetrahydronaphthalin standard, with 4.80 min. retention time, the purity of sarin was estimated to be 81 - 84%.

GC-MS: LKB 2091, Digital PDP 11.

GC : Glascapillary column 20 m, i.d. 0.245 mm, Ucon HB 5100. Helium 28 cm/sec., Split 1:30. Temp. Column 70°C (starting temperature). Program 4°C/min. Injektor 100°C. Separator 150°C.

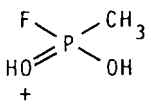
MS : Temp. of ionsource 200°C. 45 eV. Trap current 50 µA. Acceleration 3500 V.

All peaks are corresponding to those of GC and could be identified. The key-fragments M/Z 99 for sarin (A), M/Z 101 for phosphoroflu-

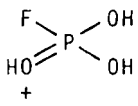
oridic acid diisopropylester (B), M/Z 83 for phosphonic acid diisopropylester (C), and M/Z 97 for [methyl-^{14}C]phosphonic acid diisopropylester (D) were identified by electron impact mass spectrometry (EIMS).

According to Tab. 1 and 2 these data are unambiguously correlated to the corresponding fragments (Scheme 5):

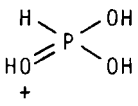
Scheme 5 Elektron Impact MS (EIMS)



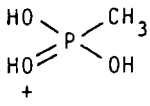
M/Z 99



M/Z 101



M/Z 83



M/Z 97

Table 1 . Results of GC- and GC-MS.

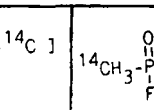
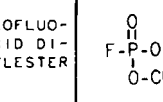
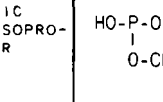
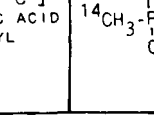
Peak	Substance		Retention		Account
	name	chem. structure	time (GC)	temp.(MS)	in %
A	[Methyl-^{14}C] sarin		1,5 min.	90 °C	87
B	Phosphorofluoridic acid diisopropylester		2,4 "	99 °C	4
C	Phosphonic acid diisopropyl ester		4,1 "	109 °C	7
D	[Methyl-^{14}C] phosphonic acid diisopropyl ester		5,2 "	113 °C	2

Table 2. Results of EIMS.

Peak	Substance	Mol. weight	Characteristic fragment M/Z (in %)
A	[Methyl-^{14}C] sarin	142,10	125(33,96), 99 (100), 81 (12,87), 79 (3,23), 43 (15,48), 42 (9,19), 41 (11,23).
B	Phosphorofluoridic acid diisopropyl ester	184,15	169(4,14), 127(73,36), 101 (100), 43(23,02), 42 (8,9), 41(14,19), 39(9,97).
C	Phosphonic acid diisopropyl ester	166,16	151(4,97),112(14,41), 109(91,94), 93(9,74), 83(100), 65(10,83), 59(12,52), 45(32,40), 43(39,76), 41(20,37).
D	[Methyl-^{14}C] phosphonic acid diisopropyl ester	180,19	165(4,92),139(9,44), 123(62,38),121(9,71), 97(100), 80(11,58), 79(27,89), 45(26,12), 43(33,74), 41(13,82), 39(5,94).

Sass und Fischer (18), with the help of chemical ionization MS (CIMS) succeeded in characterization of several organophosphorous compounds.

Synthesis of labelled [methyl-^{14}C] sarin (2*)

- Phosphorochloridous acid diisopropylester (3)

87.5 ml (1 mol) phosphortrichloride in 1200 ml ethylether at 0°C were treated with 154 ml (2 mol) isopropanol, and 165.5 ml (2 mol) of pyridine in 200 ml ethylether. After separation of the solid, the solution was evaporated and the product was purified by distillation under reduced pressure (7mm Hg) at 38-52° C. Yield 98.1g

containing about 50% of (3).

- Phosphorofluoridous acid diisopropylester (1)

60 g (0.32 mol) of phosphorochloridous acid diisopropylester were treated with 50 g (0.28 mol) of SbF_3 at 0°C (ice/water). The filtrate was distilled twice, and yielded 45g (82.3%) of the fluoride (1).

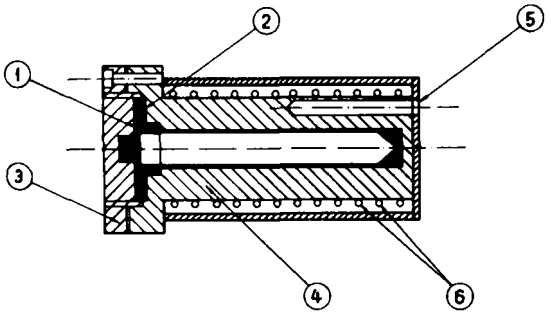
Bp_{51}= 51°C, MG 168.15, IR (carbon tetrachloride).

Microanalysis:	C	H
Calculated:	42.85%	8.39%
Found:	42.03%	8.56%

-[Methyl-^{14}C] sarin (2*)

1.0277 g (6.1 mmol) phosphorofluoridous acid diisopropylester was treated in our microreactor vessel.

Fig. 1 Microreactor for the synthesis of [methyl-^{14}C] sarin.



1 Teflon pod (5ml)
2 Teflon lid
3 Cover CrNi
4 Beaker CrNi
5 Mantle CrNi
6 Heating 300 W

with 1.3 g (9.2 mmol) methyliodide and 0.1243 g (0.9 m mol) [^{14}C-methyl]iodide (The Radiochemical CTR Ltd.,batch 192, 58 mCi/mmol). The reactor was heated with the help of a teleconducted regulator (Systag, TCU-2) and kept at 140°C for 5 hrs. The product was distilled twice under reduced pressure (Bp_{16} = 56°C) and yielded 718.9 mg (5.13 mmol) 84% of 4.651 mCi/mmol [methyl-^{14}C]sarin, re-

fered to phosphorofluoridous acid diisopropylester (1). The activity was estimated by liquid scint. (Packard, Tri-Carb. Mod. 3375). Radiochemical purity 97.75% (Gas Radiochromatography).

Synthesis of non labelled sarin (2)

- Methylphosphonic acid dichloride (4)

124.08 g (1 mol) of methylphosphonic acid dimethyl ester were added dropwise to 437.3 g (2.1 mol) phosphorous pentachloride. After one hour of stirring the mixture was heated to 80°C for another hour, and then distilled under reduced pressure. The main fraction was again distilled under ambient pressure and at 160-161°C, 76.97 g (57.9%) of methyl-phosphonic acid dichloride were yielded. Mp 33°C, MG = 132.93, and Bp = 161.5° C.

- Methylphosphonic acid difluoride (5)

120 g (2.86 mol) sodiumfluoride were added to 100 ml 1,2-Dichlorobenzene and 0.33 ml of water, and left over night. 60 g methyl phosphonic acid dichloride in 120 ml of 1,2 dichlorobenzene were added dropwise and the mixture was heated at 96°C, where distillation of the difluoride began. The product was finally distilled over a Vigreux-column. The main fraction boiling at 96-99° C yielded 37.11 g (82%), MG 100.01

- Sarin (2), (3)

6.65 g (0.05 mol) fused methylphosphonic acid dichloride, 5 g (0.05 mol) methylphosphonic acid difluoride and 13 ml dry methylenchloride boiled under reflux were treated with 5.96g (0.10 mol) of isopropanol. After evaporation at ambient temperature the product was distilled twice under reduced pressure (Bp_{7-8} = 43-44° C), and yielded 11.21 g (80.01%) of sarin, refered to methylphosphonic acid dichloride (4).

As sarin is hygroscopic we analized a sample of [methyl-^{14}C]sarin

after a period of 80 days with GC-MS. The hydrolytic product was identified according to Tab. 1 to be [methyl-^{14}C]phosphonic acid diisopropylester (D).

Stability of sarin in different solvents :

As sarin showed to be easily decomposed, we conducted the following experiments.

1. Sarin in water.
2. Sarin in Krebs-Henseleit-solution, pH 7.4.
3. Sarin in isopropanol.

- ad 1 Hydrolysis of sarin in water (Fig. 2)

Fig. 2

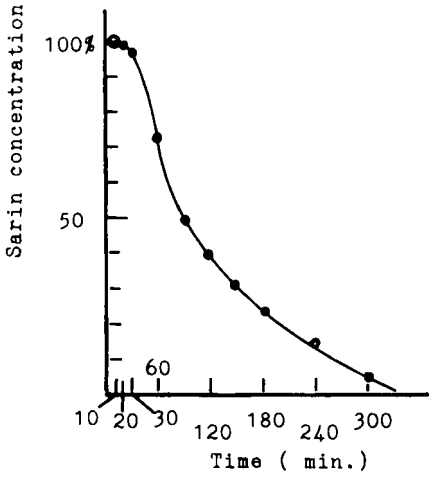
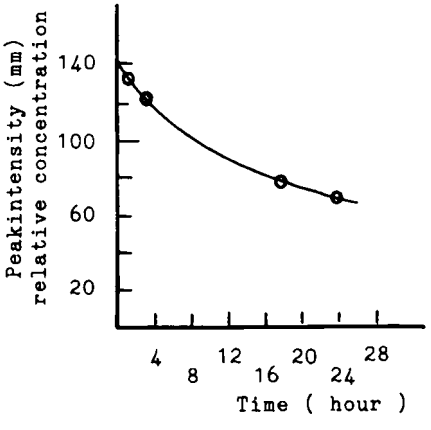


Fig. 3



Temperature 20 °C , concentration 12.1 mg/ml water. The hydrolysis depends on the starting concentration, and with a half-life of 1.5 hrs. sarin is almost completely decomposed within 6 hrs.

- ad 2 Hydrolysis of sarin in Krebs-Henseleit-solution (Fig. 3)

Temperature 20° C, concentration 2 mg/ml solution. Under these conditions the half-life was estimated to be 24 hrs.

- ad 3 Sarin in isopropanol.

Temperature 20°C, concentration 2 mg/ml isopropanol. No change of the concentration of sarin was noted, even after a period of four months.
Based on these results all our samples were stored under isopropanol.

DISCUSSION

The characterization of a receptor was first accomplished with labelled substances (6-8). In order to obtain reliable results the labelled substance has to fit the receptor in the closest possible conformation. The substance must be stable in respect of chemical decomposition as well as radiolysis. Substances with long half-life and high radioactivity are suited best. In this respect we had to consider the forementioned statements.
The aim of our synthesis was not only using the labelled sarin for receptor characterization but also for autoradiography. We analyzed our autoradiographic data for distribution as well as kinetic studies (20) of the original compounds and their metabolites. The problem of the penetration of "biological barriers" e.g.the blood-brain-barrier only could be solved with the labelled compounds.
In respect of the synthesis we had to be extremely cautious not only because of the radioactivity but we had also to take in account the high toxicity of sarin and analogs. This might be the reason why sarin is not available commercially.
To omit the risk of radioactivity as well as toxicity, and especially to exclude the risk of explosion of the usual glass equipment, we constructed a specific reactor which proved to be very convenient for the production of small amounts of labelled sarin with high purity.

Non labelled sarin was synthesized to serve as a standard of highest purity. The synthesis of non labelled sarin seems to be relatively

simple. However, the synthetic pathway of labelled sarin must be different for the following reasons:

- The one step reaction from both relatively low toxic reactants for the synthesis of the finally labelled sarin reduced the risk of poisoning. Once combined, both reactants form sarin immediately, thus labelled sarin is available at any time.
- Both reactants, phosphorofluoridous acid diisopropylester (1) and [^{14}C-methyl]iodide are relatively stable compounds and can be stored without specific precautions.
- Finally the synthetic pathway of labelled sarin proved to be more economic in respect of introduction of radioactivity to the molecule and reduced the danger of contamination.

As [methyl-^{14}C]phosphonic acid diisopropylester appears as a by-product of labelled sarin synthesis we also synthesized this compound. We found according to our animal experiments, that this by-product does neither influence autoradiography, nor toxicity of sarin (19). The problem of storage and protection from humidity in the daily use was solved by storage of all sarin samples under isopropanol.

Acknowledgements

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