PHOSPHONOMETHYLATION OF ANESTHESIN AND RADIOPROTECTANT ACTIVITY OF THE PRODUCTS

V. K. Khairullin, M. A. Vasyanina, R. Z. Musin, A. N. Pudovik, and G. G. Batulina

UDC 615.849.1.015.25].012.1

New types of compounds are continually being found to possess radioprotectant properties [9]. Phosphonic acid derivatives employed in medicine [7] have recently been found to increase the radioresistance of the body [9]. A further search in this series is clearly desirable.

We have now, for the first time, phosphonomethylated anesthesin in order to obtain esters of (p-ethoxycarbonylphenylaminomethyl)phosphonic acid. Three compounds were synthesized and their toxic and radioprotectant properties examined. As is well known, the phosphonomethylation of amines can be effected by reacting them with paraformaldehyde and trialkyl phosphites [2, 3, 6], or by the Kabachnik–Fields reaction with paraformaldehyde and dialkyl phosphites [5, 12]. It is also known that the first system is preferable for the phosphonomethylation of p-aminophenol and p-anisidine [4].

Using the first method, reaction of equimolar amounts of anesthesin, paraformaldehyde, and triethyl phosphite resulted in a complex reaction. When the reaction was carried out at 110–120°C in toluene, there were obtained 24.4% of methylene-NN'-bis-(p-ethoxycarbonylphenyl)-diamine (I), 32.6% of diethyl hydroxymethylphosphonate (II), and only 23.8% of diethyl (p-ethoxycarbonylphenylaminomethyl)phosphonate (III). In addition, a small amount of ethyl p-diethylaminobenzoate (IV) was isolated, and the toluene distillate was found to contain (IR and mass spectroscopy) ethanol. These compounds were characterized by their PMR, ^{32}P NMR, and IR spectra.

Mass spectrum of (I) (m/z [ion], relative intensity as % of maximum): 343 (0.5), 342$[M]^+$, (1.5), 179 (1.5), 178 (14), 177 (45), 166 (6), 165 (50), 150 (7), 149 (16), 148 (5), 139 (4), 137 (16), 133 (11), 132 (85), 122 (2), 121 (14), 120 (100), 105 (7), 104 (13), 103 (2), 93 (7), 92 (30), 91 (6), 78 (11), 77 (25), 76 (3), 66 (2), 65 (30), 51 (12), 45 (6), 39 (6), 29 (12), 27 (7). On electron impact, fission of the N–C bond in (I) with migration of the hydrogen of the NH group to the other nitrogen leads to the formation of two ions, $[C_2H_5OCO–C_6H_4–N=[CH_2]]^+$ with m/z 177 and $[C_2H_5OCO–C_6H_4–NH_2]^+$ with m/z 165: precise measurement of the masses of these ions (177.0782 and 165.0797) gave good agreement with the calculated values of 177.0789 and 165.0789 based on the elemental compositions $C_{10}H_{11}O_2N$ and $C_9H_{11}O_2N$ respectively. These ions subsequently readily fragmented with the ejection of the ethoxy group and the formation of strong peaks with m/z 132 and 120.

The PMR spectrum of (II) ($CDCl_3$, δ, ppm, J, Hz) was as follows: 2 q centered at 4.13 ($POCH_2$, OCH_2C), $J_{HH} = J_{HP} = 7$; t 1.33 (CH_3), J_{HH} 7; s 5.2 (OH). ^{31}P NMR spectrum of (II): $\delta_P = 24$ ppm. IR spectrum of (II), ν_{max}, cm^{-1}: 3600–3050, 3300 (OH), 1100 med (OC–C), 1040 v.s (P=O), 975 s (P–OC). PMR spectrum of (III) (CCl_4, δ, ppm: J, Hz): t 1.5 (CH_3CH_2OP), J_{HH} 7; t 1.46 (CH_3CH_2OC), J_{HH} 7; d 3.46 (CH_2P), J_{HP} 6; m 4.13–3.86 (CH_2OC, CH_2OP); s 5.03 (NH); m 6.73–6.29, m 7.90–7.56 (C_6H_4). IR spectrum of (III), ν_{max}, cm^{-1}: 3350 s (br) NH, 3060 w (=CH), 1700 v.s (C=O), 1600 v.s, 1525 s, 1440 med (C_6H_4), 1280 v.s $[C_6H_4C(O)O]$, 1100 s (PO–J), 1055 s (ω, τ, CH_3), 1030 s (P=O), 975 s (OC–C), 788 s (γ=CH), 700 med (ρCH_2), 530 med (br) [P(O)O]. Mass spectrum of IV (m/z (ion), relative intensity as % of maximum): 222 (0.90), 221 $[M]^+$ (10, 208 (0.90), 207 (13), 206 $[M–CH_3]^+$ (25), 195 (0.90), 194 (14), 193 $[M–C_2H_4]^+$ (79), 192 (6), 180 (2), 179 (23), 178 $[M–C_2H_4–CH_3]^+$ (100), 177 (0.80), 176 $[M–OC_2H_5]^+$ (5), 166 (2), 165 $[M–C_2H_4–C_2H_4]^+$ (25), 164 (16), 152 (0.90), 151 (9), 150 $[M–C_2H_4–CH_3]^+$ (45), 149 (8), 148 $[M–C_2H_4–CH_3–C_2H_6]^+$ (47), 137 (6), 135 (2), 134 (24), 131 (8), 120 $[M–C_2H_4–C_2H_4–OC_2H_5]^+$ (42), 118 (6), 106 (11), 105 (10), 104 (9), 92 (15), 91 (8), 79 (9), 78 (7), 77 (10), 66 (3), 65 (10), 45 (2), 29 (8), 27 (4). Precise measurement of the masses of the strong peaks in the mass spectrum $[M–C_2H_4]^+$ 193.1090, and $[M–C_2H_4–CH_3]^+$

A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Branch, Academy of Sciences of the USSR. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 2, pp. 191–195, February, 1988. Original article submitted September 22, 1986.

 0091-150X/88/2202-0142$12.50

178.0899 gave good agreement with the values calculated for the empirical formulas of these peaks, $C_{11}H_{15}NO_2$ 193.1090 and $C_{10}H_{12}NO_2$ 178.0868.

In the IR spectrum of the toluene fraction bp 80-110°C (3 mm), ν_{OH} absorption was present at 3150-3600 cm^{-1} for ethanol, and in the mass spectrum, in addition to the peaks for toluene at 96 (12), 92 (13), 91 (100), 65 (43), 63 (17), and 51 (20), peaks characteristic of ethanol were present at 46 (6), 45 (41), and 31 (77) [8].

$$2EtOCC_6H_4NH_2\text{-}p + CH_2O \longrightarrow \underset{I}{(EtOCC_6H_4NH\text{-}p)_2CH_2} + H_2O \qquad (1)$$

$$I + (EtO)_3P \longrightarrow (EtO)_3P(NHC_6H_4COOEt\text{-}p)CH_2NHC_6H_4COOEt\text{-}p \longrightarrow$$

$$\longrightarrow EtOCC_6H_4NHCH_2\underset{III}{P}(OEt)_2\text{-}p + [EtOCC_6H_4NHEt\text{-}p] \qquad (2)$$

$$2[EtOCC_6H_4NHEt\text{-}p] \longrightarrow EtOCC_6H_4\underset{IV}{NEt_2}\text{-}p + EtOCC_6H_4NH_2\text{-}p \qquad (3)$$

$$(EtO)_3P + CH_2O + H_2O \longrightarrow (EtO)_3P: \longrightarrow$$

$$\longrightarrow (EtO)_2\overset{+}{P}(EtO)(\overline{O}H)\text{—}CH_2OH \longrightarrow \underset{II}{(EtO)_2P(O)CH_2OH} + EtOH \qquad (4)$$

Ethyl p-diethylaminobenzoate (IV) could be formed either by disproportionation of the ethyl p-ethylaminobenzoate to the tertiary amine (IV) and anesthesin (equation 3), or by alkylation of the latter by the phosphonate ester (III). An alternative route for the formation of (III), by reaction of anesthesin with the hydroxymethylphosphonate (II) is not followed, since (II) and anesthesin fail to react on boiling in benzene for 6 h, the starting materials being recovered unchanged. Neither is (II) formed by initial hydrolysis of the triethyl phosphite to diethyl phosphite and alcohol, followed by addition of the former to paraformaldehyde. This conclusion was reached on the basis that no signal for diethyl phosphite was present in the ^{31}P NMR spectra of the reaction mixture.

A more suitable method for the phosphonomethylation of anesthesin is by use of the Kabachnik-Fields reaction, the principal products being the dialkyl (p-ethoxycarbonylphenylaminomethyl)phosphonates (III), (V), and (VI), together with (I). The reaction proceeds via the initial formation of (I), followed by reaction of this with the dialkyl phosphite, as shown in a model experiment. Even on gentle heating of (I) with an excess of diethyl phosphite, (III) is formed, as is clearly shown by the signal with δ_P 24 ppm in the ^{31}P NMR spectrum. Following removal of the diethyl phosphite under reduced pressure, n_D^{20} 1.5510.

$$I + (RO)_2PH \longrightarrow EtOCC_6H_4NHCH_2P(OR)_2\text{-}p + EtOCC_6H_4NH_2\text{-}p$$

R = Et (III), Pr (V), Bu (VI).

It is noteworthy that the reaction of paraformaldehyde with anesthesin totally prevents the reaction of paraformaldehyde with the dialkyl phosphite [1]. It should be mentioned that in addition, the reaction of anesthesin with paraformaldehyde and diethyl phosphite gave small amounts of the ethyl ester (IV). Of the (p-ethoxycarbonylphenylaminomethyl)phosphonate esters obtained, only (III) could be distilled, (V) and (VI) being characterized in the undistilled state.

The biological properties of (III), (V), and (VI) are given in Table 1. It will be seen that the toxicities (LD_{50}) of the compounds lie between 420 and 760 mg/kg. The toxic effects of (III), (V), and (VI) on animals are noteworthy in respect of the instant reaction of the body to their administration, with signs of cardiac and respiratory inadequacy (dyspnea), and symptoms of marked changes in the central nervous system (ataxia, convulsive twitching proceeding to paralysis of the muscles of the rear extremities). A few minutes following administration of the compounds, the animals adopted a spreadeagled state until death, which occurred after 30 min (compounds (III) and (IV)) or some hours (VI).

TABLE 1. Toxicity and Radioprotectant Properties of (III), (V), and (VI)

Compound	Toxicity, LD_{50} (mg/kg)	Radioprotectant properties		
		dose given, mg/kg	survival, %	ML, days
III	420	140	50±11*	14,2
V	760	253	40±11*	11,5
VI	470	157	0	7,7
Control	—	—	0	8,9

*Difference from control significant at $P \leqslant 0.01$.

High radioprotectant activity was shown by the diethyl ester (III), 50% of the experimental animals surviving when the mortality in the control irradiated mice was 100%. As the length of the hydrocarbon chain in the ester group of the substituted methyl phosphonate is increased, the radioprotectant activity decreases. For example, administration of the propyl ester of the above acid (V) resulted in 40% survival of the irradiated mice, survival with the butyl ester (VI) being 0%.

EXPERIMENTAL (CHEMICAL)

The chemical shifts of the ^{31}P nuclei were measured on a KGU-4 NMR at a frequency of 10.2 MHz, standard 85% phosphoric acid. PMR spectra were obtained on a Varian T-60 spectrometer (60 MHz) (USA), solvents $CDCl_3$ and CCl_4, internal standard TMS. IR spectra were obtained on a UR-20 spectrometer (East Germany). Droplets of the compound were compressed between KBr plates, the layer thickness not being measured. Mass spectra were obtained on a Finnigan MAT-212 instrument (USA), ionizing electron energy 50 eV, emission current 0.1 mA, temperature of inlet system 120°C. The exact values of the ion masses were found by superposition of the peaks on reference peaks of perfluorokerosene.

Reaction of Anesthesin, Paraformaldehyde, and Triethyl Phosphite. A mixture of 8.26 g (0.05 mole) of anesthesin, 1.5 g (0.05 mole) of paraformaldehyde, and 8.3 g (0.05 mole) of triethyl phosphite in 50 ml of toluene was heated at 110-120°C for 6.5 h. On cooling, the clear, colorless solution deposited colorless crystals, which were filtered off and washed on the filter with acetone to give 4.2 g (24.4%) of methylene-NN'-bis-(p-ethoxycarbonylphenyl)-diamine (I), mp 182°C, after recrystallization from alcohol 188°C (literature mp 180°C [11]). The toluene was distilled from the filtrate, 3 ml of the distillate, bp 80-110°C, being dried over anhydrous $CuSO_4$ and examined for ethanol content. Fractionation of the residue gave 2.5 g (32.8%) of diethyl hydroxymethylphosphonate (II), bp 87°C (0.007 mm Hg), d_4^{20} 1.400, n_D^{20} 1.4400 (literature values [1], bp 124-126°C (3 mm), d_4^{20} 1.1396, n_D^{20} 1.4328) and 2.5 g (23.8%) of diethyl (p-ethoxycarbonylphenylaminomethyl)phosphonate (III), bp 179-180°C (0.01 mm), n_D^{20} 1.5510, δ_P 24 ppm. Highly viscous, yellow liquid. Found, %: C 53.40; H 7.13; N 4.58; P 9.69. $C_{14}H_{22}NO_5P$. Calculated, %: C 53.33; H 7.06; N 4.44; P 9.82. From the decomposition products of the residue from distillation there was obtained 0.45 g of ethyl p-diethylaminobenzoate (IV), mp 42°C (literature values [10], mp 43°C, bp 312-314°C). Found, %: C 70.23; H 8.48; N 6.19. $C_{13}H_{19}NO_2$. Calculated, %: C 70-55; H 8.75; N 6.32.

Reaction of Anesthesin, Paraformaldehyde, and Diethyl Phosphite. In a flask fitted with a Dean and Stark apparatus were placed 8.26 g (0.005 mole) of anesthesin, 1.5 g (0.05 mole) of paraformaldehyde, and 6.9 g (0.05 mole) of diethyl phosphite, and boiled in 50 ml of benzene for 3 h. Water (0.8 ml) collected in the trap. The benzene was removed, and the residue (15.4 g) fractionated. The fraction with bp 110-120°C (0.008 mm) was crystallized, mp 81-174°C (4.6 g). The fraction boiling at 182°C (0.008 mm), 6.1 g (38.6%), n_D^{20} 1.5510, δ_P 24 ppm was (III). When the bath temprature was further increased, the residue distilled at a lower temperature than (III). The fraction bp 120-137°C partially crystallized. Filtration followed by washing the crystals with dry acetone gave 0.8 g of (IV), mp 43°C. Diethyl phosphite (0.9 g, δ_P 8 ppm) was collected in a trap cooled in liquid nitrogen.

Dipropyl (p-Ethoxycarbonylphenylaminomethyl)phosphonate (V). In a flask fitted with a Dean and Stark apparatus, a mixture of 8.26 g (0.05 mole) of anesthesin, 1.5 g (0.05 mole) of

paraformaldehyde, and 8.8 g (0.05 mole) of dipropyl phosphite was boiled in 100 ml of benzene for 16 h. Following removal of the benzene under reduced pressure, there were isolated from the residue 1 g of (I), mp 187°C, and 11.3 g (59.9%) of the ester (V), n_D^{20} 1.5280, δ_P 23 ppm. Found, %: C 55.68; H 7.74; N 4.21; P 8.82. $C_{16}H_{26}NO_5P$. Calculated, %: C 55.97; H 7.63; N 4.08; P 9.02.

Dibutyl (p-Ethoxycarbonylphenylaminomethyl)phosphonate (VI). Similarly, from 4.13 g (0.025 mole) of anesthesin, 0.75 g (0.025 mole) of paraformaldehyde, and 4.85 g (0.025 mole) of dibutyl phosphite in 50 ml of dry benzene there were obtained 1.8 g of (I), mp 187°C, and 5.6 g (60.6%) of the ester (VI), n_D^{20} 1.5120, δ_P 24 ppm. Found, %: C 57.98; H 8.26; N 3.56; P 8.18. $C_{18}H_{30}NO_5P$. Calculated, %: C 58.21; H 8.14; N 3.77; P 8.42.

EXPERIMENTAL (BIOLOGICAL)

The biological studies were carried out on 115 male mice, strain C57Bl/6, initially of age 3.5 months, weighing 20-23 g. The compounds (III), (V), and (VI) were suspended in a mixture of 0.1% carboxymethylcellulose and Tween, 49:1, and administered to the mice intraperitoneally in a volume of 0.2 ml. In determining the acute toxicity, the lethal dose of the compound causing the deaths of 50% of the animals (LD_{50}) during the seven days after treatment was found. In determining radioprotectant activity, the animals were irradiated in an IGUR-1 cesium gamma-apparatus in a dose of 8 Gy, which resulted in the death of 95-99% of the control mice in the 30 days following irradiation ($LD_{95-99}/30$). The compounds were administered to the mice in a dose of 1/3 of the LD_{50} 15 min prior to irradiation. The postradiation 30-day survival of the mice and the mean lifespan of the dead individuals (ML) were calculated.

LITERATURE CITED

1. V. S. Abramov, Zh. Obshch. Khim., 22, No. 4, 647-652 (1952).
2. V. I. Gaidai, L. A. Valitova, S. S. Krokhina, and B. E. Ivanov, Izv. Akad. Nauk SSSR, Ser. Khim., No. 3, 611-615 (1979).
3. B. E. Ivanov, S. S. Krokhina, et al., ibid., No. 12, 2773-2776 (1971).
4. B. E. Ivanov, A. B. Ageeva, S. S. Krokhina, et al., ibid., No. 1, 178-180 (1982).
5. M. I. Kobachnik and T. Ya. Medved', Dokl. Akad. Nauk SSSR, 83, No. 5, 689-692 (1952).
6. G. L. Matevosyan, A. V. Zhuravlev, R. M. Matyushicheva, and P. M. Zavlin, Zh. Obshch. Khim., 50, No. 5, 1198-1199 (1980).
7. M. D. Mashkovskii, Drugs [in Russian], 8th edition, Moscow (1977), parts 1-2.
8. V. V. Takhistov, Practical Mass Spectroscopy of Organic Compounds [in Russian], Leningrad (1977), p. 259.
9. V. G. Yashunskii and V. Yu. Kovtun, Usp. Khimii, 54, No. 1, 126-161 (1985).
10. F. L. Beilstein, Auflage, J. Springer, 14, 449.
11. O. Dimroth and K. Pfister, Chem. Ber., 43, No. 3, 2757-2767 (1910).
12. E. K. Fields, J. Am. Chem. Soc., 74, No. 6, 1528-1531 (1952).