SYNTHESIS AND CHROMATO-MASS SPECTRAL INVESTIGATION OF LABELED ANALOGS OF THE ANTITUMOR PREPARATION FOTRIN

G. V. Bornovalova, L. F. Linberg,
E. G. Tikhonova, and T. S. Safonova

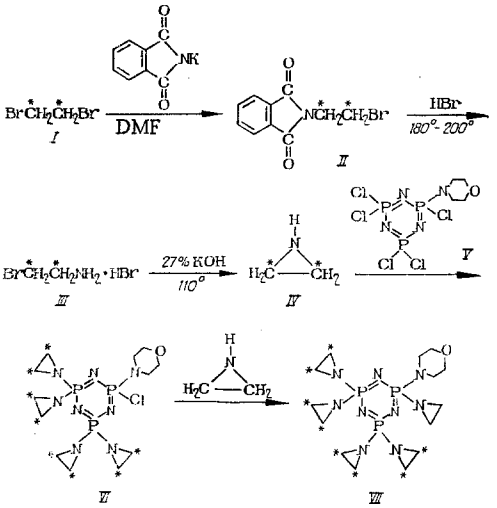
UDC 615.277.3.074:543.42.062

Fotrin (VII) is 2,2,4,4,6-pentaethylenimino-6-morpholinocyclotriphosphazatriene, an antitumor preparation used for the treatment of lymphatic leukemia, erythema, and fungoid mycoses [8].

A synthesis of the preparation labeled with the ^{32}P radioactive isotope was carried out previously with the aim of studying the distribution of fotrin in the organism and tissues and also the route of its conversion in the organism of animals. This made it possible to obtain the first data on the pharmacokinetics of fotrin and also to elucidate several of its metabolites [6, 7]. It was found that the main quantity of radioactive substances were isolated from the organism of intact rats in the first 6 h after intravenous injection. In the same period seven of its metabolites were isolated together with fotrin and were detected by thin layer radiochromatography.

The present work is devoted to the development of methods of synthesis of fotrin analogs labeled in the ethyleneimine groups with radioactive carbon ^{14}C and with deuterium and also a chromato-mass spectral investigation of the obtained compounds with the purpose of a subsequent deepened study of the pharmacokinetics and metabolism of fotrin.

For this purpose ^{14}C-ethylenimine was obtained by the known procedure of [5, 9-11] starting from 1,2-^{14}C-dibromoethane. A series of changes was applied suitable for the conditions of a radioactive microsynthesis. In particular the stage of isolating ^{14}C-ethylenimine (II) was modified. 2-Bromo-^{14}C-ethylphthalimide (II) was obtained by the interaction of 1,2-^{14}C-dibromoethane (I) with potassium phthalimide in dimethylformamide and by subsequent treatment with hydrobromic acid and then alkali was converted into ^{14}C-ethylenimine (IV). ^{14}C-6-Chloro-2,2,4,4-tetraethylenimine-6-morpholinocyclotriphosphazatriene (VI) [4] was obtained by the reaction of compound (IV) with 2,2,4,4,6-pentachloro-6-morpholinocyclotriphosphazatriene (V). Triethylamine was used to bind the formed hydrogen chloride. Replacement of the last chlorine atom in compound (VI) by an ethylenimine group was effected by treating (VI) with an excess of unlabeled ethylenimine.



S. Ordzhonikidze All-Union Scientific-Research Institute for Pharmaceutical Chemistry, Moscow. Scientific-Research Institute for Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 19, No. 5, pp. 583-588, May, 1985. Original article submitted October 14, 1983.

0091-150X/85/1905-0339$09.50

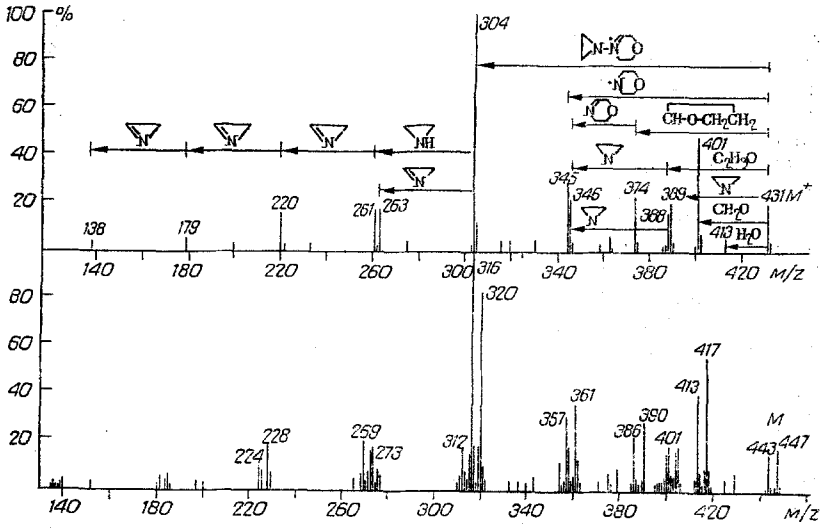
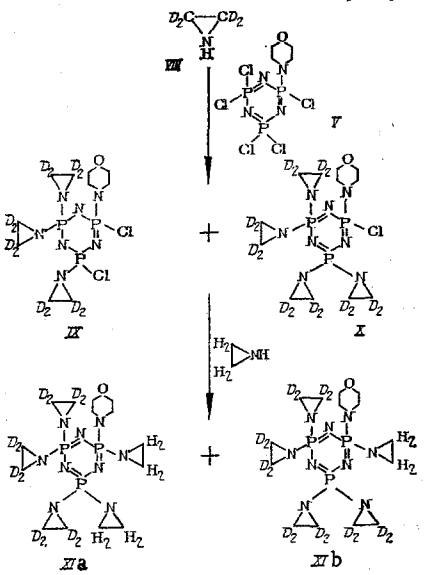


Fig. 1. Mass spectra of deuterofotrin (XIa, b). Relative intensity (%) is on the ordinate and m/z on the abscissa.

Synthesis of fotrin labeled with deuterium was carried out using tetradeutero-1,2-dibromoethane as starting material.

However from the mass spectrum of the final product, ^{2}H-fotrin (Fig. 1), it followed that together with the expected substance (XIb), containing four tetradenteroethylenimine groups ($M^{+\cdot}$ = 447), there was also an approximately equimolar quantity of compound (XIa) with three tetradeuteroethylenimine groups ($M^{+\cdot}$ = 443). This is explained by the fact that at the stage of replacing the chlorine atoms in the pentachloro derivative (V) by tetradeuteroethylenimine (VIII) an excess of (V) was taken for the purpose of a more complete use for the labeled ethylenimine (VIII). As a result of this a mixture of two intermediates (IX) and (X) was obtained which contained three and four ethylenimine groups respectively. The total yield of compounds (IX) and (X) was 91% calculated on compound (VIII). In the final stage of the synthesis of ^{2}H-fotrin, carried out in an excess of unlabeled ethylenimine, all the chlorine atoms of compounds (IX) and (X) were replaced by ethylenimine giving the mixed product (XIa + XIb) in 76% yield calculated on compound (VIII). As a result two modifications of ^{2}H-fotrin were obtained, (XIa) and (XIb), with 12 and 16 deuterium atoms at a ratio of 2:3 respectively. The presence of two groups of molecular ions in the mass spectrum of ^{2}H-fotrin was not an obstacle for subsequent chromato-mass spectral investigations of pharmacokinetics and metabolism of the preparation.



Analysis of the mass spectrum of fotrin was facilitated to a significant degree by the presence of the analog labeled with deuterium. By analogy with the breakdown of ethylenimine derivatives under the action of electron impact (EI) which was studied previously in [3] a sequential elimination of ethylenimine groups might have been expected in the case of fotrin.

TABLE 1. Intensities of Peaks of Ions Observed in the Mass Spectra of Fotrin and Its Deutero Analog (as % of main ion)

Fotrin				Deuterofotrin							
m/z	CH_4	C_4H_{10}	NH_3	m/z	CH_4	C_4H_{10}	NH_3	m/z	CH_4	C_4H_{10}	NH_3
474	—	3,19	—	484	3,6	1,3	—	375	6,3	1,0	—
472	5,6	1,5	—	475	7,8	—	—	362	8,4	1,7	—
461	6,2	—	—	472	11,5	—	—	361	10,5	2,8	—
460	26,5	—	—	450	—	2,0	16,5	360	4,2	0,6	—
434	5,5	2,4	2,7	449	14,2	19,0	100	359	3,6	0,4	—
433	41,8	19,4	18,5	448	57,3	100	17,8	358	8,9	1,8	—
432	97,6	100	100	447	25,2	19,5	4,7	357	16,3	1,8	—
431	60,4	7,9	—	446	18,9	7,0	13,9	356	4,7	0,4	—
430	53,4	4,6	—	445	20,5	15,5	78,2	355	3,6	0,2	—
429	1,6	—	—	444	78,9	85,0	11,7	354	4,7	0,5	—
417	1,6	—	—	443	32,1	12,5	—	321	3,6	0,5	—
414	2,5	0,6	—	442	21,3	3,3	—	320	16,8	3,5	7,3
413	9,9	2,1	0,6	441	6,3	1,2	—	319	4,7	0,7	—
406	—	—	2,7	440	3,6	1,0	—	317	5,2	0,8	—
403	6,2	1,2	—	429	5,5	2,2	—	316	27,8	4,4	7.4
402	9,0	2,6	—	425	5,7	1,6	—	315	5,2	0,8	—
401	48,8	7,9	1,4	422	—	—	6,5	313	3,6	0,4	—
391	3,9	—	—	418	—	1,6	6,5	312	10,5	0,9	—
390	41,8	4,6	—	417	15,8	8,0	8,2	281	3,6	—	—
389	100	28,3	—	416	5,2	1,6	—	273	5,2	0,7	—
388	17,5	3,6	1,3	415	4,7	0,7	—	272	5,2	0,7	—
387	6,2	1,1	—	414	5,5	1,3	—	271	3,6	0,3	—
386	2,1	0,4	—	413	21,0	5,7	6,0	269	10,5	1,2	—
375	5,8	—	—	412	5,2	0,9	—	265	3,6	0,2	—
374	13,9	0,7	0,6	406	7,3	3,2	—	229	4,2	0,5	—
373	—	2,3	—	405	39,5	22,0	4,3	228	9,4	1,1	—
363	7,2	—	—	404	13,1	5,1	—	227	3,1	0,3	—
359	2,3	—	—	403	6,3	1,4	—	225	5,2	—	—
348	2,5	—	—	402	16,8	4,9	—	224	7,3	—	—
347	4,1	—	—	401	100	2,9	4,7	207	7,8	—	—
346	16,9	0,8	—	400	24,7	4,9	—	184	4,2	0,4	—
345	19,7	3,1	0,6	399	5,2	1,1	—	183	3,1	—	—
344	—	2,6	—	398	7,8	1,2	—	181	6,3	0,4	—
305	6,9	1,1	—	397	33,1	4,6	—	180	4,2	0,3	—
304	46,5	6,2	—	396	6,3	0,7	—	152	3,6	—	—
263	6,9	—	—	391	3,6	0,7	—	136	4,2	—	—
261	7,2	—	—	390	6,8	1,8	3,4	116	3,1	—	—
220	9,7	—	—	389	3,6	0,4	—	88	9,9	—	—
179	4,1	—	—	387	4,2	0,2	—	86	13,1	—	—
101	—	—	5,9	381	7,8	—	—	85	5,2	—	—
				379	4,2	1,2	—	70	7,8	—	—

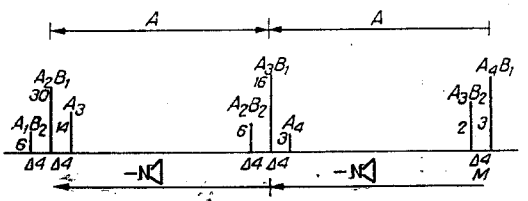


Fig. 2. Calculated ratios of isotopic peaks obtained on elimination of one and two ethylenimine groups from the molecular ion of deuterofotrin.

However the presence of a morpholine substituent made an appreciable contribution to the character of the fragmentation. This is particularly marked for deuterofotrin (XI). As already mentioned there were two forms of the labeled fotrin molecule, viz., A_3B_2 and A_4B_1 in a ratio 2:3, where A is the number of ethylenimines labeled with deuterium and B contains only hydrogen atoms. In the case of the elimination of one ethylenimine group under conditions of their equivalence a collection of fragments 2.3/5 A_2B_2, 3.4/5 A_3B_1, and 3.1/5 A_4 must be obtained where the numerical coefficients reflect the probability of their formation. Similarly on removal of a second ethylenimine group a collection of A_1B_2, A_2B_1, and A_3 ions must be observed in the ratio 6:30:14. Graphically the picture shown in Fig. 2 was obtained. From a comparison of this spectrum with the mass spectrum of deuterofotrin it follows that fragments $[M-18]^+$, $[M-30]^+$, $[M-57]^+$, $[M-86]^+$ were formed without loss of deuter-

ium atoms from the molecule (breaking away of particles of H_2O, CH_2O, $\dot{C}H{-}O{-}CH_2CH_2$, and the morpholine nucleus). The formation of the ions $[M-42]^+$, $[M-43]^+$, $[M-85]^+$, and $[M-127]^+$ was accompanied by ejection of a labeled ethylenimine group but to a significantly lesser extent than follows from calculation. It was therefore possible to draw the conclusion that the initial suggestion of the equivalence of the ethylenimine groups was wrong and the unlabeled ethylenimine group located beside the morpholine ring was more readily split off. This is seen particularly clearly in the example of the formation of the $[M-127]^+$ fragment. This group of ions (m/z 312, 316, 320) must have a ratio of 6:16:3 under conditions of equivalence of fission of any ethylenimine group. However fragment A_4 (m/z 320) clearly predominates but fragment A_2B_2 is less than calculated. It therefore follows that the single unlabeled ethylenimine group is eliminated more readily. One of the most logical explanations of this fact is the hypothesis that the morpholine nucleus together with the ethylenimine group located beside it is split off as a single particle. It was possible to check this hypothesis by taking the spectra of daughter ions [1]. The subsequent fragmentation of fotrin as might have been expected was caused by the sequential fission of ethylenimine fragments.

With the aim of determining the stability of fotrin to various forms of chemical ionization (CI), spectra were obtained using methane, isobutane, and ammonia as gas reactants (see Table 1). From a comparison of the obtained spectra it is evident that the peak of the pseudomolecular ion $(M + H)^+$ was the most intense. In the CI–CH_4 spectrum in addition a pseudomolecular ion $(M + C_2H_5)^+$ was observed. As might have been expected the stability of fotrin towards ionization grew on going from methane to ammonia and in the CI–NH_3 spectrum only an $(M + H)^+$ ion was present. This made it possible to use the mild CI–NH_3 method in the search for the products of metabolism of this preparation in complex mixtures.

A chromato-mass spectral study of fotrin and its labeled analogs showed that the preparation possessed volatility sufficient for gas chromatographic analysis (retention time was 15.7 min on a column 1.8 m × 2 mm with 3% SE-30 at a temperature of 270°C and helium supply rate 20 ml/min). The formation of decomposition products was not observed under these conditions.

EXPERIMENTAL

A Varian MAT-212 mass spectrometer was used in the work and was linked through a platinum capillary with a Varian Aerograph 3700 chromatograph fitted with a glass column 1.8 × 2 mm with 3% SE-30 on Chromosorb W-HP. Carrier gas was helium (20 ml/min). Injector temperature was 270°C, ionizing voltage 70 eV (EI), 200 eV (CI), emission current 1 mA (EI), 0.5 mA (CI), and temperature of ion source 250°C.

β-Bromo-^{14}C-ethylphthalimide (II). Potassium phthalimide (5.8 g:0.0308 mole), dibromo-^{14}C-ethane (6.54 g:0.0348 mole), and dimethylformamide (6.7 ml) were heated on a water bath to 60-70°C. An exothermal reaction began at this temperature with a sharp jump in temperature of the reaction mixture to 120°C. At the end of heat evolution the reaction mixture was cooled to room temperature, carbon tetrachloride (35 ml) was added, and water (130 ml) was poured in. The aqueous layer was separated and extracted with carbon tetrachloride (15 ml × 2). The organic layers were combined, washed with 0.2 N NaOH (30 ml), and with water (30 ml). After distillation of carbon tetrachloride (II) (4.14 g:52%) was obtained as a white crystalline substance of mp 76-80°C (79-81°C according to literature data).

β-Bromo-1,2-^{14}C-ethylamine Hydrobromide (III). A mixture of β-bromo-^{14}C-ethylphthalimide (II) (4.14 g:0.0163 mole) and concentrated HBr (density 1.49:20 ml) was boiled under reflux at 180-200°C for 2 h, cooled, cold water (30 ml) added, and the solid filtered off. The filtrate was evaporated to dryness on a water bath. Compound (III) (3 g:90%) was obtained as a dark orange crystalline substance with mp 155-160°C (from ethanol).

^{14}C-Ethylenimine (IV). A solution of β-bromo-1,2-^{14}C-ethylamine hydrobromide (3 g:0.015 mole) in 18% KOH solution (8 ml) was poured dropwise onto dry KOH (3 g) heated to 110°C. Ethylenimine was distilled off with water by this means into a receiver cooled to −10°C containing dry KOH (20 g). Separation of an organic layer occurred in the receiver which was distilled again into a receiver with dry KOH (1-2 granules). Compound (IV) (0.1 ml:13.5%) was obtained with bp 56-57°C (56°C according to literature data).

6-Chloro-2,2,4,4-^{14}C-tetraethylenimino-6-morpholinocyclotriphosphazatriene (VI). A solution of 2,2,4,4,6-pentachloro-6-morpholinocyclotriphosphazatriene (0.5 g) in methylene chloride (2 ml) was added dropwise to a mixture of ^{14}C-ethylenimine (0.25 ml) (0.15 ml un-

labeled ethylenimine was added to the obtained ^{14}C-ethylenimine) and triethylamine (0.6 ml) in methylene chloride (2 ml) cooled to −15°C. The reaction mixture was kept at −15°C for 30 min, then at 18°C for 12 h. The precipitate of triethylamine hydrochloride was filtered off and the reaction mixture evaporated on a rotary evaporator. Compound (VI) (0.5 g:94%) was obtained with mp 169-170°C (from ethyl acetate, 170-172°C according to literature data of [2]).

2,2,4,4,6-^{14}C-Pentaethylenimino-6-morpholinocyclotriphosphazatriene (VII). Unlabeled ethylenimine (0.6 ml:225% excess) was added at one stroke to a solution of compound (VI) (0.5 g: 0.00123 mole) in methylene chloride cooled to −15°C. The reaction mixture was kept at 18°C for 72 h. The precipitate of ethylenimine hydrochloride was filtered off, the filtrate was evaporated to dryness, and crude ^{14}C-fotrin (0.508 g) was obtained. After chromatographic purification on a column of silica gel (height 250 mm, diameter 10 mm) ^{14}C-fotrin (0.4:78%) was obtained with mp 119-122°C (120-123°C according to literature data of [8]).

Analysis of the purity of the obtained product was carried out by TLC on Silufol plates in the system methanol—acetone (9:1) visualized with Dragendorf reagent. The R_f value of ^{14}C-fotrin agreed with the R_f of the pharmacopoeia preparation and was 0.25-0.30.

LITERATURE CITED

1. O. S. Anisimova, L. F. Linberg, and Yu. N. Sheinker, Mass Spectrometry in the Investigation of Drug Metabolism [in Russian], Moscow (1978), p. 87.
2. A. A. Kropacheva, L. E. Mukhina, N. M. Kashnikova, et al., Chemistry and Application of Organophosphorus Compounds [in Russian], Moscow (1962), p. 1372.
3. L. F. Linberg, Yu. N. Sheinker, and T. S. Safonova, Khim. Geterosikl. Soedin., No. 2, 263-267 (1975).
4. L. E. Mukhina and A. A. Kropacheva, Zh. Obshch. Khim., 32, 521-525 (1962).
5. A. Murray and L. D. Williams, Organic Synthesis with Isotopes: Part 1, Compounds of Isotopic Carbon, Interscience (1958).
6. V. A. Ovchinnikova, P. P. Filatov, V. A. Chernov, et al., Khim.-farm. Zh., No. 11, 18-23 (1977).
7. A. S. Singin, G. V. Bornovalova, and T. S. Safonova, in: Pharmacokinetics and Metabolism of Drugs [in Russian], Pt. 7, Moscow (1978), pp. 58-64.
8. V. A. Chernov, O. B. Lytkina, S. I. Sergievskaya, et al., Farmakol. Toksikol., No. 4, 365-368 (1959).
9. S. Gabriel, Chem. Ber., 21, 566-570 (1888).
10. J. Sheehan, J. Am. Chem. Soc., 72, 2786-2789 (1950).
11. A. Wilson, K. Reeves, and G. Drake, J. Am. Chem. Soc., 73, 3522-3527 (1951).