Chemistry of Natural Compounds, Vol. 35, No. 2, 1999

SYNTHESIS OF ^{14}C-RADIOACTIVELY LABELED SAMPLES OF BATRIDEN

V. N. Sumin,[a] A. D. Sakhibov,[b] N. I. Baram,[a] and A. I. Ismailov[a]

UDC 615.021.1: 547.565

Samples of batriden (a drug based on gossypol) radioactively labeled with ^{14}C in different parts of the molecule have been synthesized. Pharmacokinetic investigations of the ^{14}C-batridens have enabled the mechanism of the action of the drug to be elucidated.

For an understanding of the mechanism of the action of a drug, knowledge of its distribution not only at the tissue level but also at the cellular, subcellular, and molecular levels is necessary. In order to conduct pharmacokinetic investigations of batriden (an immunosuppressive drug based on gossypol) we have synthesized ^{14}C-radioactively labeled samples of batriden: ^{14}C-batriden-1 was obtained by the interaction of [^{14}C]gossypol containing the ^{14}C label in an aldehyde group with barbituric acid; and ^{14}C-batriden-2 was synthesized from pharmacopeial gossypol and [^{14}C] barbituric acid. Thus, two radioactive forms of ^{14}C-batriden containing the ^{14}C label in different parts of the molecule (Fig. 1) were obtained.

The results of the radiochemical analyses performed (TLC and radiochromatography [1]) witnessed the radiochemical purity of the ^{14}C-batriden-1 and the presence of the radioactive impurity [^{14}C]barbituric acid in the sample of ^{14}C-batriden-2 (Fig. 2). In order to obtain radiochemically pure ^{14}C-batriden-2, the sample was subjected to additional purification.

As is known, barbituric acid is readily soluble in hot water [2, while batriden is insoluble in water. Starting from this, the freeing of the ^{14}C-batriden-2 from contaminating [^{14}C]barbituric acid was achieved by repeated lengthy washing in which the labeled substance was stirred with hot (80°C) distilled water. To avoid flotation of the drug, ethanol was used at the beginning of the purification process [3]. The fall in the radioactivity of the wash waters is shown in Fig. 3.

After the preparation had been freed from the radioactive impurity in this way, the ^{14}C-batriden-2 was again subjected to radiochromatographic analysis. Zonal analysis of the radiochromatograms of the ^{14}C-batriden-2 showed the purity of the radioactively labeled compound (Fig. 2, c).

The results of a study of the distribution of ^{14}C-batriden-1 over the organs and tissues of experimental animals (mice) have been published elsewhere [4, 5]. The considerable accumulation and prolonged circulation of ^{14}C-batriden-1 in the organs of the immunity system (liver, lymph nodes, thymus, and spleen) have been of fundamental importance in the characterization of batriden as an immunosuppressor.

In an investigation of the excretion of ^{14}C-batriden-1 from the organism it was found that about 20% of the intraperitoneally injected drug underwent biotransformation. To elucidate details of this process we have now performed pharmacokinetic experiments with ^{14}C-batriden-2 and have made a comparative analysis of the pharmacokinetic indices of ^{14}C-batriden-1 and ^{14}C-batriden-2. No fundamental difference was found. On this basis, it was concluded that the metabolic transformations of batriden take place not through the splitting off of the barbituric acid from the gossypol but as a result of the biotransformation of the gossypol and (or) the barbituric acid with retention of the link between them.

Pharmacokinetic investigations of ^{14}C-batriden conducted at the subcellular and molecular levels of murine hepatocytes and blood plasma revealed pronounced membranotropic properties of the drug. Thanks to its high affinity for lipids, batriden accumulates in the lipid layers of the cell membranes (mitochondrial, plasmatic) and, binding with their protein components, including enzymes localized in these membranes, exerts an influence on their functional activity.

---

a) A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent. b) Institute of Immunology, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 185—187, March-April, 1999. Original article submitted April 12, 1998.

0009-3130/99/3502-0165$22.00 ©1999 Kluwer Academic/Plenum Publishers

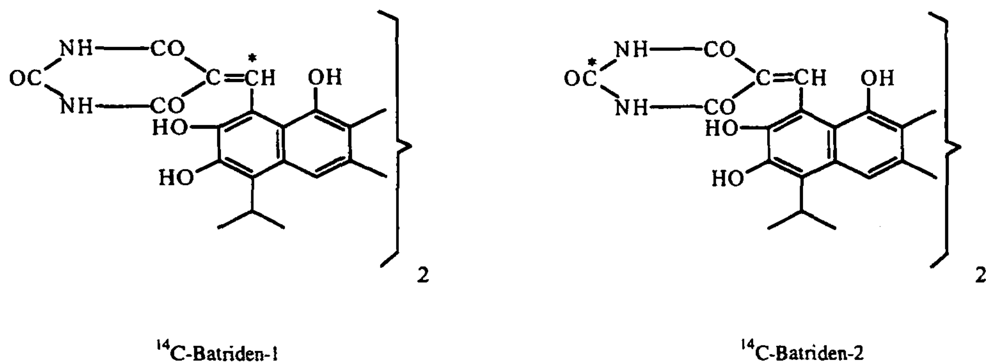

Fig. 1. Radioactively labeled forms of batriden.

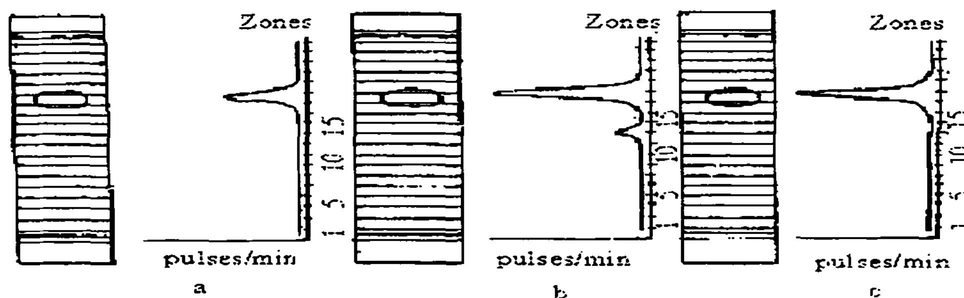

Fig 2. Radiochromatography of samples of ^{14}C-batridens: *a*) ^{14}C-batriden-1; *b*) ^{14}C-batriden-2 before purification; *c*) ^{14}C-batriden-2 after purification.

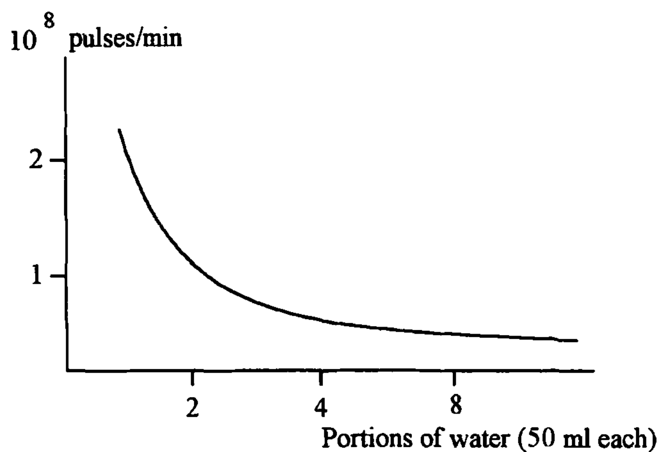

Fig. 3. Radioactivity of the wash waters in the purification of ^{14}C-batriden-2.

EXPERIMENTAL

The individuality of the compounds synthesized was checked by TLC on Silufol UV-254 plates in the systems: 1) acetone—benzene—acetic acid (1:9:0.6), and 2) acetone—toluene (7:3). The radiochemical purity of the radioactively labeled samples of batriden was determined by zonal analysis (20 zones 5 mm wide) of their thin-layer chromatograms. The radiometry

of the samples was conducted on a Rack-Beta 2 radiospectrometer (LKB, Sweden) using ZhS-8 scintillation liquid.

[^{14}C]Gossypol was obtained by the method described in [6]. Its specific radioactivity (sp. r.) was 1.2 μCi/mg. TLC: R_f 0.55 in system 1, 0.9 in system 2.

Synthesis of ^{14}C-Batriden-1. Barbituric acid (0.3 g) was boiled in ethanol (10 ml) for 3 h. In parallel, 0.3 g of [^{14}C]gossypol was dissolved in 7 ml of ethanol at 80°C. The solutions were combined, and heating was continued. The resulting ^{14}C-batriden-1 was filtered off and washed successively with ethanol and diethyl ether. Yield 0.27 g, sp. r. 0.6 μCi/mg. TLC in system 2: R_f 0.7; radiochromatography: R_f 0.7.

Synthesis of ^{14}C-Batriden-2. [^{14}C]Barbituric acid (46 mg, with a total radioactivity of 6 mCi) was boiled in 70% ethanol (5 ml) until it had dissolved completely. In parallel, 0.82 g of barbituric acid was boiled with 28 ml of ethanol for 3 h, and 1.0 g of gossypol was dissolved with heating in 24 ml of ethanol. To this gossypol solution was added the solution of [^{14}C]barbituric acid and, after the mixture had been boiled for 30 min, that part of the barbituric acid which had dissolved in the ethanol. After prolonged heating, a precipitate of ^{14}C-batriden-2 formed. The product was filtered off and was washed with ethanol, hot distilled water, and diethyl ether. Yield 0.86 g (60.6%); specific radioactivity 4.4 μCi/mg. TLC in system 2: R_f 0.7; radiochromatography: R_f 0.7, 0.4).

Purification of ^{14}C-Batriden-2. ^{14}C-Batriden-2 (0.86 g) was finely ground in an agate mortar, transferred to a conical flask, and suspended in 4 ml of ethanol, and then 50 ml of distilled water was added. With constant stirring, the mixture was heated in a water bath at 80°C for 30 min and was then centrifuged at 3000 rpm for 5 min, after which the supernatant liquid was taken off and its radioactivity was measured. Another 50 ml of distilled water was added to the residual solid and the process of purifying the drug was repeated. In this way we obtained eight 50-ml portions of wash waters and determined their radioactivities. The residual ^{14}C-batriden-2 was dried at 80°C. The yield of product was 0.7 g (81.7%), sp. r. 3.8 μCi/mg. TLC in system 2: R_f 0.7; radiochromatography: R_f 0.7.

The pharmacokinetic investigations of the ^{14}C-batridens were conducted in experiments on random-bred white mice weighing 20—22 g. The drug was injected intraperitoneally in a single dose of 100 mg/kg. The distribution of radioactivity over the organs and its elimination from the organism were studied by methods described previously [4]. The isolation of subcellular fractions of a liver homogenate and the fractionation of labeled macromolecules of murine hepatocytes and blood plasma were carried out as described in the literature [7].

REFERENCES

1. T. Robert, *Radiochromatography*, Elsevier Scientific, Amsterdam (1976) [Russian translation, Mir, Moscow (1981), p. 46].
2. *Chemical Reagents and Preparations*, A Handbook (ed. A. I. Zitser), Goskhimizdat, Moscow (1953).
3. A. I. Bondarenko, *Farmatsiya*, **4**, 31 (1986).
4. V. I. Sumin, A. I. Ismailov, and N. I. Baram, *Khim. Prir. Soedin.*, 810 (1997).
5. A. D. Sakhibov, B. Z. Kasymov, V. I. Sumin, N. I. Baram, and A. I. Ismailov, *Khim.-farm. Zh.*, 1990, dep. No. 3087-V90.
6. C. M. Lyman, M. M. Trant, J. T. Cronin, and F. N. Odell, *J. Am. Oil Chem. Soc.*, **46**, 100 (1968).
7. O. Yu. Abakumova, *Modern Methods in Biochemistry* [in Russian], Meditsina, Moscow (1977), p. 338.