DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

PRINCIPLES OF KSIMEDON PRODUCTION TECHNOLOGY

V. S. Reznik,[1] A. A. Muslinkin,[1] A. N. Shirshov,[1] N. A. Spiridonova,[1] N. G. Pashkurov ,[1,2] V. D. Akamsin,[1] and É. A. Gurylev[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 35, No. 12, pp. 28 – 31, December, 2001.

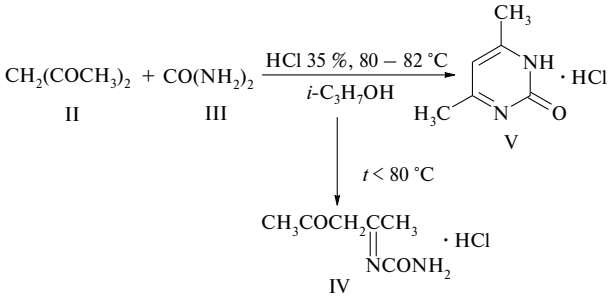
Original article submitted July 9, 2001.

Ksimedon [1], representing 1-(2-oxyethyl)-4,6-dimethyl-1,2-dihydropyrimidin-2-one (I), was originally obtained in 1965 within the framework of a systematic investigation into the synthesis and biological activity of N-(ω-oxoalkyl)-oxodihydropyrimidines – nonglycoside analogs of pyrimidine nucleosides [2, 3]. Among a number of synthesized substances, compound I showed the best combination of properties and was selected for clinical testing. Initially, ksimedon was considered as comparable in activity with 6-methyluracil (metacil). However, subsequent investigations showed that these drugs are pharmacologically different: ksimedon has a pronounced action upon the immune system, while metacil shows no such activity.

According to the results of clinical tests, ksimedon was recommended for commercial production and application in medicine as an antiburn agent with immunostimulant action [4]. However, subsequent pharmacological and clinical investigations showed that the therapeutic spectrum of ksimedon extends far beyond treating burns. The preparation has proved to be effective in cases of various nosologic forms of surgical disorders in adults and children [5]. This drug was recommended for accelerating wound healing in cases of large injury areas [6], for the prophylaxis of postoperative pyoinflammatory complications [7], and for the treatment of trophic ulcers [8]. According to experimental and clinical data, ksimedon produces positive effects in the treatment of osteomyelitis [9], osteoporosis, gastric and duodenal ulcers, chronic gastritis, hepatitis, and leukopenia induced by chemo-, x-ray-, and radiotherapy of malignancies. It was also reported that the drug was effective in the complex therapy of patients with acute pneumonia [10] and tuberculosis [11].

In connection with the good prospects for the broad application and increased commercial production of ksimedon, we have conducted a chemico-technological investigation of the process of synthesis, isolation, and purification of the parent compound. The results of this study are presented below.

We have developed a special three-stage technology for the synthesis of ksimedon based on readily available reagents: acetylacetone (II) and carbamide (III). The first stage is essentially the reaction of condensation of II and III in the presence of HCl, which was originally described in [12]. This reaction leads to the formation of 2-oxy-4,6-dimethylpyrimidine hydrochloride (V):

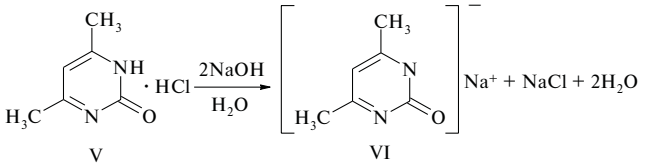


Although carrying out this reaction presents no difficulties, deviation from the indicated temperature regime results in the formation of a certain amount of the incomplete condensation product IV. Replacing ethanol (used in the original process [12]) by isopropyl alcohol somewhat increases the yield of product V.

In the second stage, compound V is converted into sodium salt VI. Previously [2], we described the two-step synthesis of compound VI, whereby V is converted first into a free base by treatment with NaOH and then into a Na salt by interaction with sodium alcoholate. Now we have established that compound VI is simply and conveniently synthesized in

[1] Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, Kazan, Tatarstan, Russia.
[2] Deceased.

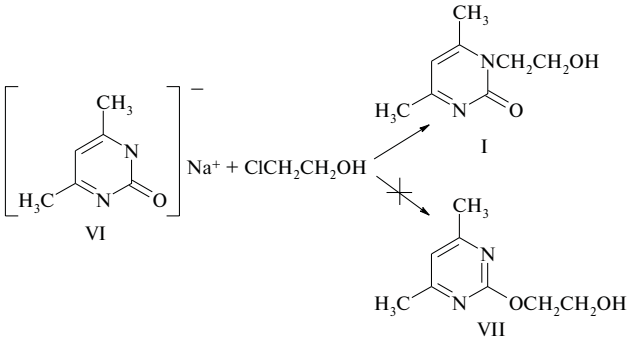
one step by treating V with two moles of NaOH in a strictly controlled amount of water, followed by separating and drying the target product:



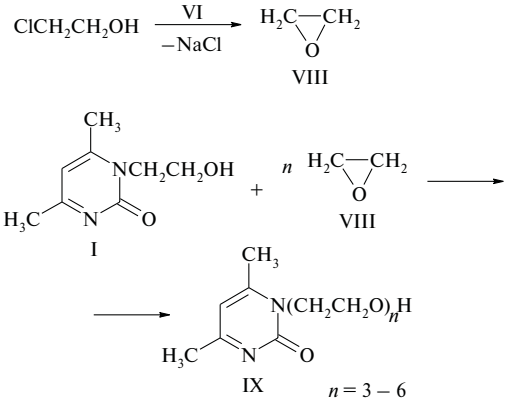
The results of this reaction depend to a certain extent on the ratio of compound V and NaOH (as can be seen from Table 1, the alkali should be taken in a small excess).

In order to purify the intermediate product VI from the unavoidable NaCl admixture, the product was recrystallized from an aqueous isopropyl alcohol (IPA) solution. In the case of a 25 – 30% aqueous IPA solution, the filtration of the hot solution is hindered by rapid crystallization. Increasing the IPA concentration to 35 – 40% renders the process technologically feasible and leads to a 4 – 5% increase in the content of the target compound in the mixture, although the yield is still as low as 30 – 35%. For this reason, taking into account that the admixture of NaCl does not hinder the third stage in the synthesis of ksimedon, we simplified the process and carried out this stage using dry salt VI without additional purification.

In the final stage, the alkylating agent ethylene chlorohydrin (ECH) interacts with salt VI according to the scheme



This reaction yields only product I (the formation of an O-alkylation product VII was never observed). The reaction proceeds smoothly in an alcohol medium (methanol, IPA, *n*-propanol), as well as in aqueous solutions and DMF. However, both isolation of I from the reaction medium containing water or DMF and subsequent purification are complicated by the side reactions leading to the formation of a considerable amount of oligomers (IX) appearing as viscous liquids:



The formation of ethylene oxide (VIII) takes place in virtually all cases, reaching a maximum when compound I is synthesized in an aqueous medium using 2-oxy-4,6-dimethylpyrimidine potassium salt and minimum, when the reaction is conducted in an anhydrous alcohol using a sodium salt (VI). As for the interaction between VIII and I, this process is most significant both in aqueous media and in DMF. Compound VIII also reacts to a certain extent with salt VI to form I, but the contribution of VIII to ksimedon is probably more significant. In a special experiment, salt VI was reacted with compound VIII in a molar ratio of 1 : 2 in DMF at 60 – 80°C, which led to the formation of a mixture containing 10 – 25% of initial salt VI, 25 – 35% of I, and 30 – 40% of oligomers IX.

An important factor complicating the process of crude ksimedon purification until obtaining the required white color is related to the formation of otherwise insignificant (below 0.03%) impurities rendering the technical product I cherry-red, cream, or yellow. The most intense coloration is observed when ksimedon is synthesized in an aqueous medium or DMF. The formation of these impurities is related to the acid properties of methyl groups in compound IV and I: these groups are capable of detaching protons in an alkaline medium and entering various condensation reactions with the formation of unidentified colored products.

The most difficult technological problem consists in purifying the technical product I so as to obtain a substance of

TABLE 1. Effect of Reagent Ratio on the Yield and Purity of Sodium Salt VI

Molar ratio [V]/[NaOH]	Yield of VI, %	Parent substance content, %
1 : 1.96 (equimolar)	85.0	91.7
1 : 2.06 (+5 %)	87.5	94.9
1 : 1.86 (–5 %)	84.0	87.7

TABLE 2. Effect of Compound I Concentration in Solution on the Results of Chromatographic Purification of Ksimedon

Concentration of I in $CHCl_3$, kg/liter	Purified product		
	Yield, %	Parent substance content, %	Color
1.0	61.0	99.7	Pink
0.9	58.9	99.8	White with pink tint
0.8	58.8	99.5	White with slight pink tint
0.7	60.0	99.3	–"–

pharmacopoeial quality. In attempts to reach this goal by recrystallization, we tried using various solvents such as acetonitrile, methyl ethyl ketone, dichloromethane, and their mixtures, as well as ethyl acetate – pure and in mixtures with methanol, ethanol, and IPA. We have also attempted at using various schemes for extracting VI or I from technical product or its solutions in chloroform and other solvents. Neither of these methods allowed us to obtain a product of the required quality.

Finally, we succeeded in obtaining ksimedon of pharmacopoeial purity by chromatographic filtration of technical I through a column with neutral aluminum oxide, followed by precipitating the target product with acetone and drying. The chromatographic procedure simultaneously removed both residual salt VI and colored viscous impurities and yields a product containing not less than 99% of the target parent compound with a yield of 30% and above (calculated for the initial compound III). However, ksimedon obtained by this method exhibited coloration (from yellow to pink) on storage even in the dark. This color could be eliminated by repeated purification of I using the same chromatographic filtration through Al_2O_3 (with unavoidable loss of the product). As can be seen from Table 2, the lower the concentration of the initial colored chloroform solution of I, the closer the final product color approaches the desired white.

In order to solve the problem of product I coloration, we tried to precipitate ksimedon in the presence of various discolorating additives (H_2O_2, activated charcoal, zinc powder, 25% aqueous NH_3, etc.). These agents were introduced both in the initial chloroform solution or in acetone used to precipitate the product. The most successful variant of "whitening" was offered by treating the initial solution of I with a mixture of aqueous H_2O_2 and NH_3 solutions. This treatment eliminates the pink color; the yellow tint (if present) can be eliminated by additional treatment with an aqueous chloramine B solution.

With a view to the prospect of using an injection form of ksimedon, it was of interest to develop a method for additionally purifying I so as to obtain a substance forming absolutely colorless transparent aqueous solutions. This was achieved by recrystallization of I from acetonitrile in the presence of activated charcoal. Table 3 presents the results of a series of experiments, all of which yielded transparent aqueous solutions. However, an absolutely colorless preparation was obtained only if the initial weight of I did not exceed 40 – 50 g and the amount of activated charcoal was about 1.7 – 2.6% of the total charge. If the weight of I was 50 – 100 g or higher, the recrystallized product (albeit still forming transparent solutions) acquired a yellowish tint and needed additional recrystallization (unavoidably reducing the pure product yield).

In the final stage of our investigation, we have tried (on a laboratory scale) a more convenient method of synthesis, which excludes isolation and drying of the intermediate sodium salt VI – the "bottleneck" of the technological scheme described above. According to the modified scheme, hydrochloride V treated with an alkali in a methanol solution converts into sodium salt VI, which (without isolation) is reacted in methanol with ECH so as to form I. It was found that, if the alkali is taken in equimolar amount, the content of salt VI in technical product I is rather high (up to 20% and above). A more satisfactory result was obtained using the alkali in a 20% excess, whereby the content f sodium salt VI in technical I was about 12%. Dissolution in chloroform and purification by filtration through aluminum oxide completely removed salt VI and increased the yield of pharmacopoeial ksimedon to 40 – 45% (calculated for the initial carbamide).

Based on the results of these investigations, a technological process has been developed by which the parent substance of ksimedon (according to Pharmacopoeial Clause FS 42-3754–99) is now synthesized at the Arbuzov Institute of Organic and Physical Chemistry (Kazan). Using this parent

TABLE 3. Effect of Recrystallization Conditions on the Yield and Purity of Ksimedon

Experiment	Amount (g) and fraction (%) in charge						Parent substance content		Yield of pure I, %
	ksimedon		activated charcoal		acetonitrile				
	g	%	g	%	g	%	initial, %	final, %	
1	20	17.4	1.0	0.9	94	81.7	95.05	99.2	83.4
2	20	17.2	2.0	1.7	-"-	81.1	-"-	99.1	82.2
3	20	17.1	3.0	2.6	-"-	80.3	-"-	99.4	72.0
4	40	-"-	6.0	-"-	188	-"-	99.3	99.5	72.6
5	80	-"-	12.0	-"-	376	-"-	"	99.7	81.6
6	400	17.2	40	1.7	1880	81.1	95.05	99.2	80.3
7	-"-	17.1	60	2.6	-"-	80.3	98.9	99.9	83.3
8	-"-	17.0	70	3.0	-"-	80.0	96.3	99.2	81.0
9	-"-	16.9	80	3.4	-"-	79.7	-"-	98.0	84.1
10	-"-	16.5	150	6.2	-"-	77.3	-"-	98.7	77.0
11	320	24.2	Repeated without activated charcoal		1020	75.8	98.0	99.1	91.3

substance, "TatKhimFarmPreparaty" Company produces ksimedon tablets (according to Pharmacopoeial Clause FS 42-3774–99). Both products are registered at the State Pharmacopoeial Committee (reg. Nos. 93/287/7 and 94/34/11).

EXPERIMENTAL PART

2-Oxy-4,6-dimethylpyrimidine hydrochloride (V). A 63-liter enameled jacketed reactor equipped with a stirrer and a bottom outlet valve was charged (with stirring) with 21.1 liters (16.6 kg) of IPA, 4.23 kg (70.4 mole) of carbamide and 7.2 liters (7.0 kg, 70.0 mole) acetylacetone. To this mixture heated to 80 – 82°C was gradually (over 1.5 – 2 h) added from a measuring tank 9.5 liters (11.2 kg, 107.4 mole) of 35% hydrochloric acid. The temperature of the reaction mixture was initially controlled by the rate of dosing; in the later stage, by switching on the heater. Then the reaction mass was stirred on weak boiling for 2.5 – 3 h and allowed to stand overnight. Finally, the mass was filtered and the deposit on filter was washed with 2.0 – 2.5 liters IPA and dried in air to constant weight (15 – 20 h); yield, 8.5 kg (77%); m.p., 255 – 256°C (analytical sample recrystallized from aqueous IPA). The data of elemental analyses agree with the results of calculation according to the empirical formula $C_6H_8N_2O \cdot HCl$.

2-Oxy-4,6-dimethylpyrimidine sodium salt (VI). A solution of 2.24 kg NaOH in 8.3 liters of distilled water was charged into a 20-liter Simax glass reactor equipped with a paddle stirrer, immersed heating coil, and bottom outlet valve. Then the heater was switched on and 4.4 kg of V was added by portions with stirring such that the temperature would not rise above 95°C. When V was fully added, the mixing was continued with heating to 98 – 105°C until complete dissolution of the solid phase, after which the hot solution was immediately discharged into a stainless steel (or porcelain) crystallization vessel and allowed to stand overnight. The crystallized mass was transferred onto a suction (Nutsch) filter and thoroughly squeezed. Then the product was dried in air for 15 – 20 h and then in vacuum (20 – 30 Torr) at 100 – 110°C; yield, 3.5 kg (85%); m.p., 255 – 256°C; content of the parent compound (by data of potentiometric titration with 0.1 M aqueous HCl), 91.7%. The data of elemental analyses agree with the results of calculation according to the empirical formula $C_6H_7N_2ONa$ (analytical sample recrystallized from aqueous IPA).

(2-Hydroxyethyl)–4,6-dimethyl-1,2-dihydropyrimidin-2-one (I). A 20-liter Simax glass reactor equipped with a paddle stirrer, immersed heating coil, and bottom outlet valve was charged (with stirring) with 11.0 liters (8.7 kg) of methanol and 3.8 liters (4.56 kg, 56.6 mole) of ECH. To this mixture heated to 40 – 50°C was gradually (over 7 – 8 h) added, by portions with stirring, 6.0 kg (34 mole) of sodium salt VI. Then the temperature was increased to 68 – 70°C (weak boiling) and the mixture was stirred for 18 – 20 h. Upon cooling, the reaction mass was filtered and the deposit on filter was washed with 4.0 liters of methanol. The filtrate was poured into a 25-liter enameled jacketed reactor equipped with a stirrer, descending-flow cooler, and a bottom outlet valve, after which methanol was distilled off in vacuum (20 – 30 Torr). The process is continued (with stirring) until complete removal of methanol, with the rate of distillation controlled by the rate of the heating agent flow in the jacket. Then 18.0 liters of acetone was added to the reactor (at a temperature not exceeding 40°C) and the mixture was stirred about 0.5 h at 20 – 25°C. The reaction mass was filtered and the deposit on the filter was washed with 5 – 6 liters of acetone.

The obtained technical product was dried in air for 7 – 8 h to obtain 3.4 kg of a dry substance; 1.0 kg of this product was dissolved in 10.0 liters of chloroform. The solution was filtered and passed through a Simax glass column filled with aluminum oxide (~2.5 kg). Then the column was washed with 5 liters of chloroform and the obtained solution was combined with the first eluate. The entire solution was evaporated until the first signs of crystallization were visible (at a volume of about 3 – 4 liters), poured with stirring into a reactor containing 20 liters of acetone, and allowed to stand for 10 – 15 min. The obtained suspension was filtered, and the deposit on the filter was washed with acetone (1.0 liter) and dried in air to constant weight to obtain 0.66 kg of pharmacopoeial ksimedon; m.p., 139 – 141°C (reported m.p., 134.5 – 135.5°C [3]); total yield in this stage, 2.26 kg (43.2%).

Ksimedon synthesis without isolation and purification of sodium salt (VI). A 1-liter flask equipped with mechanical stirrer, thermometer, and reflux cooler was charged with 0.5 liter of methanol. Then 100.0 g (2.5 mole) of NaOH was dissolved with stirring, 160.5 g (12.9 mole) of hydrochloride V was added by portions, and the mixture was boiled with stirring for 12 h. Then 110 ml (132 g, 1.6 mole) of ECH was added and boiling was continued for 19 – 20 h, after which the reaction mass was cooled and filtered. The deposit on the filter was washed with 50 ml of methanol and the filtrate was evaporated at a reduced pressure (20 – 30 Torr) on a water bath. The residue was treated with 400 ml of acetone. The precipitated technical product was separated by filtration, washed on filter with acetone, and dried to constant weight in air to obtain 110 g (65.5 %) of technical ksimedon. Purification on an aluminum oxide column as described above yields 72.0 g (42.9%) of a pharmacopoeial product; m.p., 138.5 – 139.5°C. The data of elemental analyses agree with the results of calculation according to the empirical formula $C_8H_{12}N_2O_2$.

Ksimedon purification by recrystallization from acetonitrile with activated charcoal. A suspension of 400 g of I, 70 g activated charcoal, and 2.4 liters of acetonitrile was heated to boiling, filtered hot, and allowed to stand for 4 h. The precipitated product was separated by filtration, washed with acetone, and dried to constant weight in air to obtain a

yellowish coarse-crystalline product; yield, 320 g (80.0%); m.p., 140 – 141°C (see Table 3, experiment no. 8).

Ksimedon purification by repeated crystallization from acetonitrile. Ksimedon purified as described above (320 g) was dissolved in acetone (1.3 liter). Immediately upon complete dissolution of the product, the flask is placed into a bath with ice-cold water. After keeping under these conditions for 3 h, the precipitate was separated by filtration, washed with acetone, and dried to constant weight in air to obtain a slightly yellowish fine-crystalline product; yield, 292 g (91.0%); m.p., 142 – 143°C (see Table 3, experiment no. 11).

REFERENCES

1. S. G. Izmailov, V. S. Reznik, A. A. Muslinkin, et al., *Medica*, No. 4, 35 – 37 (1998).
2. V. S. Reznik and N. G. Pashkurov, *Izv. Akad. Nauk SSSR*, *Ser. Khim.*, No. 9, 1613 – 1617 (1966).
3. V. S. Reznik and N. G. Pashkurov, *Izv. Akad. Nauk SSSR*, *Ser. Khim.*, No. 6, 1323 – 1329 (1968).
4. USSR Inventor's Certificate No. 1,685,454; *Byull. Izobret.*, No. 39 (1991).
5. S. M. Gorbunov, *Anabolic Preparations. Autoallodermoplastics in Clinical Practice* [in Russian], Poligrafizdat, Kazan (1980), pp. 26 – 30.
6. G. A. Izmailov, S. G. Izmailov, V. Yu. Tereshchenko, et al., in: *Pharmacology and Toxicology of Organofluoric and Biologically Active Substances* [in Russian], Mari Poligrafizdat, Ioshkar-Ola (1966), pp. 72 – 73.
7. S. G. Izmailov and I. F. Sharafislamov, *Prophylaxis of Postoperation Pyoinflammatory Wound Complications in Abdominal Surgery* [in Russian], State Technical University, Kazan (1996), p. 192.
8. RF Patent, No. 2019176; *Byull. Izobret.*, No. 17 (1994).
9. RF Patent, No. 2073514; *Byull. Izobret.*, No. 5 (1997).
10. RF Patent, No. 2054937; *Byull. Izobret.*, No. 6 (1996).
11. RF Patent, No. 2088231; *Byull. Izobret.*, No. 24 (1997).
12. G. M. Kosolapoff and C. H. Roy, *J. Org. Chem.*, **26**, 1895 (1961).