STRUCTURE OF STIZOLIN – A SESQUITERPENE LACTONE FROM *Stizolophus balsamita*

M. N. Mukhametzhanov, V. I. Sheichenko,
A. I. Ban'kovskii, and K. S. Rybalko

UDC 547.913.5+547.473.2

The epigeal part of Stizolophus balsamita (Lam.) Cass. ex Takht. – Centaurea balsamita Lam., – which has been studied repeatedly, is widely used in folk medicine in fever, and externally in anginas and herpes [1]. From the epigeal part of this plant an alkaloid with the composition $C_{15}H_{23}NO_5$, which has been called stizolophin [1], and a flavonoid, 5-O-β-D-glucosyl-3-O-methylquercetin [2], have been isolated.

From the leaves and flower heads of S. balsamita collected in Central Asia, we isolated [3] a new sesquiterpene lactone – stizolin – with the composition $C_{15}H_{20}O_4$, mp 184.5-186.5° C (decomp., from benzene), $[\alpha]_D^{20} - 30.46°$ (c 2.68; chloroform). In the present paper we describe the determination of the structure of stizolin.

The IR spectrum of stizolin has absorption bands at 3560 cm^{-1} (OH), and 1757 and 1650 cm^{-1} (carbonyl of an α-methylene-γ-lactone ring). The NMR spectrum ([4], p. 506) has the signals of methyl protons: singlets at 1.12 ppm – CH_3 at an epoxide – and 1.57 ppm – CH_3 at a double bond; there are also signals of an epoxide proton (doublet at 2.83 ppm), of a lactone proton (triplet at 4.09 ppm), and a proton in the geminal position to a hydroxy group (4.02 ppm); in the 5.13-ppm region is located the signal of a vinyl proton, and two quartets in the 6.4-6.52 region are the signals of an exocyclic methylene group conjugated with the lactone carbonyl. The small distance between these signals (0.12 ppm) shows that there is a substituent in the β position to the double bond [5, 6]. The presence of an α-methylene-γ-lactone ring is also confirmed by the UV spectrum (λ_{max} 202 nm, ε 12,662).

Stizolin (I) contains one OH group (Chugaev-Tserevetinov [Zerewitinoff]), which is readily acetylated with the formation of substance (II) having the composition $C_{17}H_{22}O_5$. In the NMR spectrum (Fig. 1), the distance between the signals of the protons of the exocyclic methylene is increased (doublets at 5.71 and 6.30 ppm), which shows the presence of the OH group in stizolin in the β position to the exocyclic double bond; a sextet at 4.47 ppm is the signal of a hemiacetyl proton; a quartet at 4.19 ppm that of a lactone proton; a singlet at 1.97 ppm that of an acetyl methyl; and singlets at 1.75 ppm and at 1.22 ppm those of a vinyl methyl group and of an epoxide methyl group, respectively.

When stizolin was treated with CrO_3 in pyridine, the hydroxyl was oxidized to an oxo group giving compound (III) with the composition $C_{15}H_{18}O_4$.

The hydrogenation of (I) over a Pt catalyst in ethanol, in which 1 mole of hydrogen was consumed, formed dihydrostizolin (IV), $C_{15}H_{22}O_4$, and, from this, acetyldihydrostizolin (V), $C_{17}H_{24}O_5$, and dehydrodihydrostizolin (VI), $C_{15}H_{20}O_4$, were obtained. IR spectrum: λ_{max} 226, 302 nm (ε 1251, 276). The maximum at 226 nm (ε 1251) cannot be ascribed to an α,β-unsaturated ketone, in the first place because (VI) gives a positive Zimmerman reaction for $CO-CH_2$ and in the second place because, according to the NMR spectrum, in the neighborhood of the vinyl proton there are not two but three protons (see below).

Substances containing no conjugated systems of double bonds have been described in the literature, but in the UV spectrum they give a low-intensity absorption maximum characteristic for an ethylene or a carbonyl group [7, 8].

All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 405-412, July-August, 1971. Original article submitted February 9, 1971.

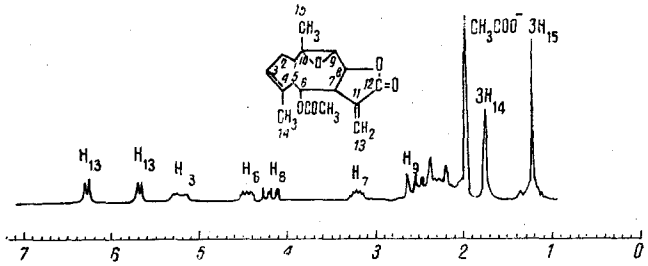


Fig. 1. NMR spectrum of acetylstizolin (100 MHz, $CDCl_3$).

Dihydrostizolin (IV) was obtained by the reduction of stizolin with $NaBH_4$, which is again in agreement with literature information on the reduction of an exocyclic methylene conjugated with a lactone carbonyl [9].

The hydrogenation of stizolin over a Pt catalyst in glacial acetic acid took place with the consumption of two moles of hydrogen and the formation of the tetrahydro derivative (VII), $C_{15}H_{24}O_4$, and the acetyl derivative (VIII), $C_{17}H_{26}O_5$.

The oxidation of tetrahydrostizolin with chromic anhydride in acetic acid led to dehydrotetrahydrostizolin (IX), $C_{15}H_{22}O_4$. The NMR spectra of tetrahydrostizolin and its derivatives lack the signals of the protons of a methyl group at a double bond and of a vinyl proton, in addition to which an additional signal of the CH_3-CH protons appears (doublet at 1.06 ppm).

The fourth oxygen atom is in the epoxide function. Attempts to open the epoxide ring with BF_3 and NaOH according to literature methods [7, 10] did not give the desired results; resinified products were formed. When (IX) was treated with 1% H_2SO_4 solution [11] with subsequent very careful neutralization of the reaction products with $NaHCO_3$, we obtained a crystalline product – the sodium salt of a trihydroxy acid (X) with the composition $C_{15}H_{25}O_6Na$.

When (IX) was treated with 0.5% in $NaHCO_3$ solution, a crystalline product with mp 170-173° C deposited, the composition and IR spectrum of which were identical with those of the starting material. According to the NMR spectrum, this substance is a mixture of (IX) and its epimer at C_{11}.

To establish the carbon skeleton of stizolin, we used the dehydrogenation method. For this purpose, dihydrostizolin (IV) was reduced with lithium tetrahydroaluminate, and the liquid glycol (XI) formed, with the composition $C_{15}H_{28}O_4$, was dehydrogenated in the presence of selenium at 290-310° C for 30 min. Chamazulene (XII) and linderazulene (XIII) were isolated from the reaction products. It is known that azulenes can be obtained not only by the dehydrogenation of bicyclic sesquiterpenes (of the type of guaiane and ambrosane) but also by the dehydrogenation of some germacranolides – monocyclic compounds [7, 11, 12]. With the composition of stizolin $C_{15}H_{20}O_4$, mol. wt. 264 (by mass spectrometry), only a monocyclic structure will be characteristic of the presence of two double bonds and one epoxy group for stizolin. Consequently, stizolin has a germacrane carbon ring. Thus, the chemical and spectroscopic facts given above permit two structural formulas to be proposed for stizolin (I) and (Ia) – which are in harmony with the NMR spectra of stizolin and its derivatives that have been obtained (Table 1). In particular, in the NMR spectrum of stizolin and acetylstizolin (see Fig. 1), the signal of the lactone proton forms a triplet with narrow components at 4.09 ppm ($J_{8,9}$ = 8 Hz; $J_{8,7}$ = 7 Hz) and a quartet at 4.19 ppm ($J_{8,9}$ = 9 Hz; $J_{8,7}$ = 7 Hz), respectively. It follows from this that there are only two protons (at C_7 and C_9) in the vicinal position to the lactone proton (at C_8). This conclusion is in harmony with the existence of a doublet of one proton unit at 2.58 ppm (J = 9 Hz) in acetylstizolin and 2.83 ppm in stizolin characterizing an epoxide proton (at C_9). A quartet with broadened components at 5.13 ppm (J = 11 and 4 Hz) in stizolin and at 5.24 ppm in acetylstizolin relate to the signal of a vinyl proton. This is located at C_3-C_4, since the signal of this proton is a quartet; furthermore, the signal of the hemiacetyl proton in (II) is a sextet at 4.47 ppm ($J_{6,7}$ = 4 Hz; $J_{6,5}$ = 9.5 Hz; $J_{6,5}$ = 4 Hz). Consequently, in the environment of this proton there are not two but three protons.

TABLE 1. Features of the NMR Spectra of Stizolin and Its Derivatives

Compound	$H_{13'}$	H_{13}	H_{11}	H_3	H_5	H_8	H_9	Acetyl-methyl	Other protons
I*	6,40q 6,52q ($H_{13,13'}$=2, $H_{13,7}$=3,5; $H_{13',7}$=3) ‡	1,12s**	1,57s	5,13q (11; 4)	4,02	4,09t ($H_{8,9}$=8, $H_{8,7}$=7)	2,83d (8)		H_7 d 3,19 m
II*	5,71d 6,30d (3,5)	1,22s	1,75s	5,24q ($H_{3,2}$=5; $H_{3,2'}$=11)	4,47sx ($H_{6,7}$=4; $H_{6,5}$=9,5, $H_{6,5'}$=4)	4,19q ($H_{8,9}$=9; $H_{8,7}$=7)	2,58 d (9)	1,97	H_7 3,21 m
IV*	1,39d (7)	1,19 s	1,66s	5,17 (5; 11)	3,75	3,85t ($H_{8,9}$=8,5, $H_{8,7}$=9,5)	2,60 d (8,5)		H_{11} 2,90 o ($H_{11,7}$=11)
V†	1,4 d (7)	1,27s	1,80s	5,26q ($H_{3,2}$=11; $H_{3,2'}$=5)	4,93q ($H_{6,5}$=$H_{6,5'}$=$H_{6,7}$=7,5)	4,01t ($H_{8,7}$=9,5 $H_{8,9}$=8,5)	2,69 d (8,5)	2,08 s	
VII†	1,50 d (7)	1,43s	1,06 d (6)		4,09	3,89t ($H_{8,9}$=9, $H_{8,7}$=10)	3,01 d (9)		H_{11} 2,79 o ($H_{11,7}$=11)
IX*†	1,27 d (7)	1,31s	1,10 d (6)			4,02t ($H_{8,9}$=9,5)	2,97 d ($H_{9,8}$=9)		H_7 3,31 q ($H_{11,7}$=12,5, $H_{7,8}$=9,5)

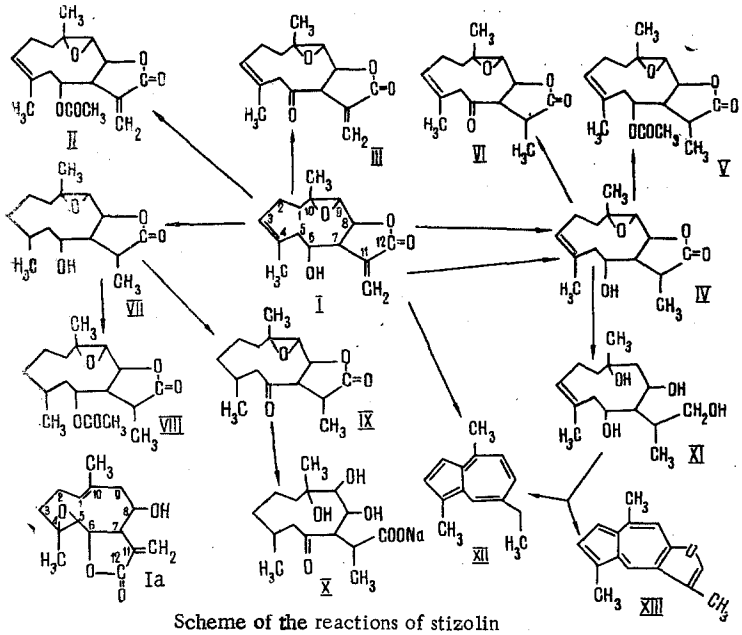
*Relative to HMDS; †relative to TMS; ‡coupling constant (Hz) with the proton the number of which is shown in brackets; **s) singlet; d) doublet; t) triplet; m) multiplet; q) quartet; o) octet; sx) sextest.

In favor of the assumption that the double bond is located at C_3-C_4 is also the absence of equidistant spacings between the lines in the spectrum of the signals of the vinyl and hemiacetyl protons.

The ease of formation of azulene in the dehydrogenation of (I) and (XI) is in favor of structure (Ia), but in this case, artemazulene or a mixture of artemazulene and linderazulene could be obtained in the dehydration of (XI) if the neighboring, nonlactone hydroxyl took part in the closure of the ring [13]. We obtained only linderazulene, which excludes the participation of the neighboring hydroxyl in the appearance of the furan ring and shows the position of the lactone ring at C_7-C_8. Thus, we propose structure (I) for stizolin.

When stizolin was treated with conc. NH_4OH, a glassy product containing nitrogen and giving a positive reaction with alkaloid reagents was obtained. It is known [14] that sesquiterpene lactones containing an α-methylene-γ-lactone ring give adducts with ammonia.

When chromatographed on neutral Al_2O_3 (activity grade IV), the resins after the extraction of stizolin from the ether–ethanol (1 : 1) fraction yielded a second lactone with the composition $C_{20}H_{26}O_7$, mp 152-153.5° C (from ethanol), identical with stizolicin – a sesquiterpene lactone from St. coronopifolius (Lam.) Cass. [15].



Scheme of the reactions of stizolin

EXPERIMENTAL

Isolation of Stizolin. The epigeal part of St. balsamita (Lam.) collected on June 28, 1967 in the region of the Kurdai mountains (Kazakhstan) (5 kg) was steeped in distilled water (75° C) for 1 h three times. The aqueous extract was treated with chloroform three times, and the solvent was distilled off. The dark viscous mass obtained was dissolved in 3 ml of ethanol, and then ether was added in portions of 5-10 ml. First the resin dissolved, but on the subsequent addition of ether a cream-colored crystalline precipitate deposited (yield 0.01%). TLC on neutral Al_2O_3 (activity grade IV) in the benzene–ethanol (9 : 1) system showed two spots with R_f 0.44 and 0.29 (the revealing agent was a 0.5% solution of $KMnO_4$ in 0.5% H_2SO_4). The combined lactones were washed with ethyl acetate five times, stizolin being obtained in the form of a white crystalline powder with mp 179-183° C giving, on TLC, a single spot with R_f 0.51 [benzene–ethanol system, (9 : 1)]. It was then recrystallized three times from benzene, mp 184.5-186.5° C (decomp. $[\alpha]_D^{20}$ – 30.46° (c 2.68; chloroform).

Found %: C 68.35; 68.33; H 7.69; 7.62. $C_{15}H_{20}O_4$. Mol. wt. 276 (cryoscopically), 264 (mass spectrometrically). M_{lab} 0.34, 0.36. Calculated%: C 68.16; H 7.63. Mol. wt. 264.31. M_{lab} 0.38.

IR spectrum: ν_{max} 3560 (OH), 1757 and 1650 cm^{-1} (carbonyl of an α-methylene-γ-lactone).

Acetylstizolin (II). A solution of 0.5 g of stizolin in 10 ml of pyridine was treated with 5 ml of acetic anhydride and heated in the water bath at 50-60° C for 1 h. After cooling, a 15-fold amount of water was added and the reaction product was extracted with chloroform. The chloroform extract was washed with 3% HCl solution and then with water to neutrality, and the chloroform was distilled off. A liquid product with the composition $C_{17}H_{22}O_5$ was obtained which crystallized on being washed with ether. It was recrystallized from ethanol; it underwent a change at 157° C but did not melt below 300° C.

The microanalytical figures for acetylstizolin and the subsequent derivatives correspond with the calculated values.

IR spectrum: ν_{max} 1768 cm^{-1} (γ-lactone), 1740 cm^{-1} ($OCOCH_3$), and 1655 cm^{-1} (C = C).

Dehydrostizolin (III). A mixture of 0.1 g of stizolin, 5 ml of pyridine, and 0.1 g of CrO_3 was left at 0° C for 18 h and was then diluted with water, and the reaction product was extracted six times with benzene–ether (1:1). The extract was washed with 3% HCl and with water; distillation of the solvent yielded colorless crystals of (III), $C_{15}H_{18}O_4$, mp 219-221° C (decomp.; from ethanol). IR spectrum: ν_{max} 1775 cm^{-1} (γ-lactone), 1712 cm^{-1} (C = O), and 1665 cm^{-1} (C = C). UV spectrum: λ_{max} 205, 303 nm (ε 11,864, 521).

Dihydrostizolin (IV). The hydrogenation of 0.5643 g of stizolin was performed in 50 ml of ethanol in the presence of 0.1 g of PtO_2 (according to Adams) until the absorption of hydrogen ceased, 1 mole of hydrogen having been consumed. The catalyst was filtered off and the alcohol was evaporated off. Colorless crystals of (IV) deposited; $C_{15}H_{22}O_4$, mp 198-200° C (from ethanol). IR spectrum: ν_{max} 3550 cm^{-1} (OH), 1757 cm^{-1} (γ-lactone).

Treatment of Stizolin with $NaBH_4$. In small portions, 2.5 g of $NaBH_4$ was added to a solution of 0.5 g of stizolin in 50 ml of CH_3OH. After 40 min, the solution was diluted with water (200 ml) and acidified with 20% sulfuric acid to pH 1 and was then treated with chloroform; the chloroform extract was washed with water until it gave a neutral reaction to universal indicator. Evaporation of the chloroform yielded a crystalline product, which was recrystallized from benzene (mp 195-198° C); it was identical with (IV).

Acetyldihydrostizolin (V). A mixture of 0.4 g of dihyrostizolin, 4 ml of acetic anhydride, and 8 ml of pyridine was heated at 50-60° C for 1 h, and after cooling the reaction mixture was diluted with water. Crystals of (V), $C_{17}H_{24}O_5$, mp 180-182° C (from ethanol) deposited. IR spectrum: ν_{max} 1770 cm^{-1} (γ-lactone), 1735 and 1250 cm^{-1} ($OCOCH_3$).

Dehydrodihydrostizolin (VI). A solution of 0.7 g of CrO_3 in 10 ml of 90% CH_3COOH was added to a solution of 0.7 g of dihydrostizolin in 10 ml of 90% CH_3COOH, and the mixture was left at +5° C for 12 h and was then diluted with water and the reaction product was extracted with chloroform. The chloroform extract was washed with 5% $NaHCO_3$ solution and with water to neutrality. After the chloroform had been eliminated a liquid was obtained, and on the addition of ether this formed colorless crystals with mp 187-190° C (from ethanol), $C_{15}H_{20}O_4$. IR spectrum: ν_{max} 1780 cm^{-1} (γ-lactone), 1700 cm^{-1} (C = O in a medium-sized ring), and 1640 cm^{-1} (C = C). UV spectrum: λ_{1max} 226 nm (ε 1251); λ_{2max} 302 nm (ε 276).

Tetrahydrostizolin (VII). The hydrogenation of 0.5046 g of stizolin was performed in 30 ml of glacial acetic acid in the presence of 0.101 g of PtO_2 (according to Adams), 2 moles of hydrogen being consumed. The platinum was filtered off, and the mixture was diluted with a fivefold amount of water and treated with small portions of Na_2CO_3 to give a neutral reaction. The hydrogenation product was extracted with chloroform, and the extract was washed with water to neutrality and the chloroform was distilled off. This gave colorless crystals with the composition $C_{15}H_{24}O_4$, mp 180-182° C (from ethanol). IR spectrum: ν_{max} 3542 cm^{-1} (OH) and 1785 cm^{-1} (γ-lactone).

Acetyltetrahydrostizolin (VIII). A solution of 0.1 g of tetrahydrostizolin was treated with acetic anhydride as described in the preparation of (V). Colorless crystals with the composition $C_{17}H_{26}O_5$, mp 169-170.5° C (from ethanol) deposited. IR spectrum: ν_{max} 1780 cm^{-1} (γ-lactone), 1725 and 1255 cm^{-1} ($OCOCH_3$).

Dehydrotetrahydrostizolin (IX). This was obtained in the same way as (VI). Colorless crystals, $C_{15}H_{22}O_4$, mp 199-201.5° C (from ethanol). IR spectrum: ν_{max} 1785 cm^{-1} (γ-lactone) and 1707 cm^{-1} (C = O).

Treatment of Dehydrotetrahydrostizolin (IX) with $NaHCO_3$. A solution of 0.3 g of dehydrotetrahydrostizolin in 15 ml of ethanol was treated with 0.13 g of $NaHCO_3$ in 10 ml of water and was then heated in the water bath at 80-90° C for 30 min. After cooling the mixture was diluted with water and extracted with chloroform, and the extract was washed with water to neutrality. After the solvent had been driven off, crystals deposited with the composition $C_{15}H_{22}O_4$ (IX), and these were washed with ether, mp 170-173° C (Kofler). IR spectrum: ν_{max} 1785 (γ-lactone) and 1707 cm^{-1}.

Sodium Salt of the Trihydroxy Oxo Acid (X). A solution of 0.3 g of dehydrotetrahydrostizolin in 35 ml of ethanol was treated with 17.5 ml of 1% H_2SO_4 and the mixture was shaken at room temperature for 30 min. Then it was neutralized with 5% $NaHCO_3$ solution and the solvent was distilled off. The resulting crystalline product was treated with hot benzene. The residue was dissolved in ethanol and the solution was filtered. The benzene solution deposited crystals (0.03 g) with mp 198-200° C (the initial compound). The ethanolic solution yielded colorless crystals with the composition $C_{15}H_{25}O_6Na$, darkening at 148°C. IR spectrum: ν_{max} 3400-3200 cm^{-1} (OH), 1695 cm^{-1} (C = O), and 1580 and 1410 cm^{-1} ($\overset{\overset{\displaystyle O}{\|}}{C}-O^-$).

The Glycol (XI). A solution of 1.0 g of dihydrostizolin in 50 ml of benzene was added by drops to 1 g of lithium tetrahydroaluminate in 500 ml of ether, and then the mixture was boiled for 1.5 h; after cooling, the lithium tetrahydroaluminate that had not reacted was carefully destroyed with water, and the precipitate was filtered off. Distillation of the solvent yielded a vitreous yellow product with the composition $C_{15}H_{28}O_4$. IR spectrum: ν_{max} 3360-3440 cm^{-1} (OH) and 1655 cm^{-1} (C = C).

Linderazulene (XIII) and Chamazulene (XII). The glycol (XI) (0.8 g) was dehydrogenated in the presence of 1 g of selenium at 290-310° C for 30 min. After cooling, the reaction product was extracted with petroleum ether, chromatographed on neutral alumina (activity grade II) and eluted with petroleum ether. This gave two fractions in the form of blue-violet viscous liquids with R_f 0.65 and 0.41 on TLC in petroleum ether. The first fraction was identical according to TLC with chamazulene taken as marker. The substance of the second fraction was dissolved in 20 ml of absolute ethanol and 0.01 g of trinitrobenzene was added. The yellow crystals with the composition $C_{15}H_{14}O \cdot C_6H_3N_3O_6$ that deposited were recrystallized from ethanol and dried in a vacuum desiccator for 3 days; mp 154-156.5° C.

SUMMARY

Structure (I) is proposed for stizolin – a sesquiterpene lactone from Stizolophus balsamita.

LITERATURE CITED

1. A. D. Kuzovkov, P. S. Massagetov, and R. I. Bogomazova, Zh. Obshch. Khim., 23, No. 1, 157 (1953).
2. L. M. Utkin and A. P. Serebryakova, Zh. Obshch. Khim., 34, No. 10, 3496 (1964).
3. M. N. Mukhametzhanov, V. I. Sheichenko, A. I. Ban'kovskii, K. S. Rybalko, and K. I. Boryaev, Khim. Prirodn. Soedin., 5, 56 (1969).
4. M. N. Mukhametzhanov, V. I. Sheichenko, A. I. Ban'kovskii, and K. S. Rybalko, Khim. Prirodn. Soedin., 6, 505 (1970).
5. K. S. Rybalko and V. I. Sheichenko, Zh. Obshch. Khim., 35, No. 3, 580 (1965).
6. Medicinal Plants. Chemistry [in Russian], Vol. 15 (1969), p. 168.
7. T. R. Govindachari and B. S. Joshi, Tetrahedron, 21, No. 6, 1509 (1965).
8. A. Somasekar Rao, A. Paul, Sadgopal, and S. C. Bhattacharya, Tetrahedron, 13, No. 4, 319 (1961).
9. S. M. Kupchan, J. M. Cassady, et al., J. Amer. Chem. Soc., 88, No. 22, 5992 (1966).
10. F. Sanchez-Viessa and J. Romo, Tetrahedron, 19, No. 8, 1285 (1963).
11. H. Morimoto, V. Sanno, and H. Oshio, Tetrahedron, 22, No. 9, 3173 (1966).
12. M. Suchy, V. Herout, and F. Sorm, Chem. and Ind., No. 18, 517 (1959).
13. L. Dolejs, M. Soucek, et al., Coll. Czech. Chem. Commun., 23, No. 12, 2195 (1958).
14. K. F. W. Hansen, Ber., 64, 67 (1931).
15. M. N. Mukhametzhanov, V. I. Sheichenko, K. S. Rybalko, and D. A. Pakaln, Khim. Prirodn. Soedin., 5, 125 (1969).