ethers; and 5) 0.43 g of a mixture of the 2- and 3-O-methyl ethers. The mixture of di-O-methyl ethers (0.30 g) was acetylated and chromatographed on a column of silica gel (1.6 × 30 cm), using a gradient of ethyl acetate in hexane. The yield of the acetate of the 3,4-di-O-methyl ether of (I) was 0.15 g; syrup, $[\alpha]_D^{20}$ +35.9° (c 0.7; chloroform); R_f 0.56 (hexane–ethyl acetate (1:1)). The yield of the acetate of the 2,4-di-O-methyl ether was 0.14 g; syrup $[\alpha]_D^{20}$ +50.0° (c 0.6; chloroform); R_f 0.45 (hexane–ethyl acetate (1:1)).

The mixture of 2- and 3-O-methyl ethers of (I) (0.30 g) was acetylated and was chromatographed on a column of silica gel (1.6 × 30 cm) using a gradient of dioxane in hexane. The load on the column was 0.36 g of the mixture. The yield of the acetate of the 2-O-methyl ether of (I) was 0.08 g, mp 101-102°C, $[\alpha]_D^{20}$ +55.6° (c 0.8; chloroform); R_f 0.29 (dioxane–hexane (1:4)). The yield of the acetate of the 3-O-methyl ether of (I) was 0.27 g; syrup, $[\alpha]_D^{20}$ +30.7° (c 1.8; chloroform); R_f 0.23 (dioxane–hexane (1:4)).

SUMMARY

A convenient method is proposed for the synthesis of all the methyl ethers of methyl (methyl α-D-mannopyranosid)uronate, which consists in the catalytic oxidation of the initial glycoside followed by partial methylation and the preparative separation of the methyl ethers with the aid of chromatography on silica gel.

LITERATURE CITED

1. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 429 (1983).
2. V. I. Grishkovets, A. E. Zemlyakov, and V. Ya. Chirva, Khim. Prir. Soedin., 555 (1983).
3. E. V. Evtushenko and Yu. S. Ovolov, Khim. Prir. Soedin., 132 (1987).

POLYUNSATURATED FATTY ACIDS OF THE α-LINOLENIC SERIES FROM INSULIN PRODUCTION WASTES

G. A. Frangulyan, A. V. Komkov,
E. P. Prokof'ev, V. M. Belotserkovets,
E. É. Lavut, and V. I. Panov

UDC 547.396:658.567

The following polyenic acids has been isolated from insulin production waste and have been determined quantitatively: eicosa-5Z,8Z,11Z,14Z-pentaenoic, docosa-7Z,10Z,13Z,16Z,19Z-pentaenoic, and docosa-4Z,7Z,10Z,13Z,16Z,19Z-hexaenoic acids. They were identified by PMR and ^{13}C NMR.

Higher fatty acids (HFAs) play an important physiological role in the normal vital activity of the organism. The greatest interest among them is presented by the polyunsaturated fatty acids (PUFAs) containing 20 and more carbon atoms in the chain and having more than two double bonds. One of the main functions of the PUFAs is participation in the synthesis of the prostaglandins – biologically active substances which already, today, are being used in obstetrics, gynecology, and veterinary medicine and have a great future [1-3]. PUFAs are also of independent interest, in particular, as agents for the treatment and prevention of a number of cardiovascular diseases. Thus, PUFAs of the ω-3 type and, namely, the isocosapentaenoic (I), docosapentaenoic (II), and docosahexaenoic (III) acids may prove to be promising for the prophylasis and treatment of atherosclerosis and myocardial infarct [4-8]; (I) [9-12] and (III) [13] for preventing the aggregation of thrombocytes and for the rapid dispersion of thrombi that have formed; and (I) [10, 14] and compounds including (I) and (III) with cyclodextrin [15] for lowering the cholesterol level in the blood serum.

The richest sources of PUFAs of the ω-3 type are fats of marine origin [16-18].

All-Union Scientific-Research Institute of the Technology of Blood Substitutes and Hormone Preparations, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 205-212, March-April, 1987. Original article submitted September 18, 1986.

0009-3130/87/2302-0168$12.50

TABLE 1. Compositions and Chromatographic Characteristics of Fatty Acids from the Lipid Insulin Waste

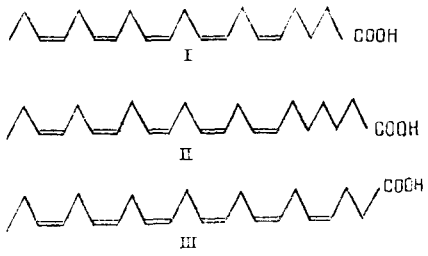
Acid	Composition of the fatty acids of the waste,%	Relative retention time*	Equivalent chain length
Palmitic (16:0)	16,0	1 00	16 00
Palmitoleic (16:1)	6,0	1,10	16,85
Stearic (18:0)	1,7	1,25	18,00
Oleic (18:1)	45,0	1,40	18,92
Linoleic (18:2)	19,0	1,55	19,80
Linolenic (18:3)	2,7	1,71	20,65
Icosenoic (20:1)	0,1	1,83	21,10
Icosatrienoic (20:3)	0,4	2,03	22,03
Arachidonic (20:4)	5,2	2,33	23,28
Icosapentaencic (20:5)	1,0	2,66	24,50
Docosatetraenoic (22:4)	0,1	2,92	25,32
Doccosapentaenoic (22:5)	1,0	3,49	26,55
Docosahexaenoic (22:6)	0,4	3,98	27,80
Myristic and other lower acids	1,6	<1	

*Relative to palmitic acid.

The search for other possible sources of PUFAs of the α-linolenic series is also urgent. In the isolation of arachidonic acid from the lipids of the pancreas — wastes from the production of insulin — we have detected three acids the R_f values of which indicated that they had a higher degree of unsaturation than arachidonic acid.

The aim of the present work was to isolate and identify these acids. The investigation showed that the acids detected were: eicosa-5Z,8Z,11Z,14Z,17Z-pentaenoic acid (I), docosa-7Z,10Z,13Z,16Z,19Z-pentaenoic acid (II), and docosa-4Z,7Z,10Z,13Z,16Z,19Z-hexaenoic (III).

The isolation of these acids was performed on the basis of a modification of a method proposed for the isolation of arachidonic acid from the same source [19] and was as follows. Extraction of the initial lipids with toluene followed by the elimination of the solvent gave a residue containing a total of 2.4% of the HFAs (I), (II), and (III) (Table 1), together with phospholipids, cholesterol, and other nonacid impurities. The HFAs were separated from the cholesterol, phospholipids, and other nonacid components on a column of silica gel. It was necessary to perform these operations since in the subsequent separation of the HFAs it is impossible to separate the PUFAs of the α-linolenic series from cholesterol. The individual PUFAs of the α-linolenic series were obtained from a mixture of HFAs with the aid of column chromatography on silica gel impregnated with silver nitrate using gradient elution by toluene containing increasing amounts of ethyl acetate.



This gave, successively, (II) with a purity of 94% in a yield of 45%; (I) with a purity of 97% and a yield of 55%; and (III) with a purity of 96% and a yield of 30%.

The elimination of a saponification stage was due to the fact that as we have shown, the acids under study are present in the free form in this waste material and are not present in the phospholipids because the latter are hydrolyzed during the production of insulin.

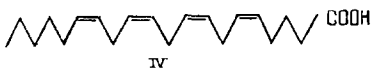
It is also undesirable to perform stages of low-temperature crystallization and high-vacuum distillation because of their inadequate efficacy and the necessity for fairly severe conditions, and also a stage of complex-formation with urea, which is accompanied by the formation of considerable wastes of the complex. It must also be mentioned that chromatography was performed without preliminary esterification of the acids, and under the conditions used it was possible to separate two acids with the same number of double bonds.

TABLE 2. ^{13}C Chemical Shifts in Polyunsaturated Acids * ($CDCl_3$, δ, ppm)

Carbon atom	Compound				
	I	II	III	IV	V
C-1	179,8 (180,4)	180,0 (180,4)	179,1 (180,4)	179,8 (180,4)	180,2 (180,4)
C-2	32,9 (33,8)	33,8 (34,2)	33,8 (34,3)	33,1 (33,8)	33,9 (34,2)
C-3	24,2 (25,1)	24,3 (24,9)	22,4 (22,4)	24,2 (25,1)	24,5 (25,0)
C-4	26,2 (26,9)	28,4 (29,0)	127,8 (128,3)	26,1 (26,9)	28,9 (29,4)
C-5	128,5 (129,0)	29,0 (29,6)	129,5 (130,2)	128,4 (129,0)	28,9 (29,4)
C-6	128,8 (129,6)	26,7 (27,1)	25,5 (25,5)	128,8 (129,6)	28,9 (29,3)
C-7	25,3 (25,7)	129,6 (130,2)	128,5 (129,2)	25,3 (25,7)	29,4 (30,0)
C-8	128,2 (129,0)	127,7 (128,4)	128,2 (128,7)	128,2 (129,0)	27,0 (27,3)
C-9	127,8 (128,5)	25,4 (25,7)	25,5 (25,6)	127,8 (128,5)	130,1 (130,5)
C-10	25,3 (25,6)	128,3 (128,8)	127,9 (128,4)	25,3 (25,6)	127,6 (128,1)
C-11	127,9 (128,5)	127,7 (128,3)	128,0 (128,4)	127,5 (128,2)	25,5 (25,7)
C-12	127,9 (128,5)	25,4 (25,6)	25,5 (25,6)	127,9 (128,7)	128,1 (128,5)
C-13	25,3 (25,6)	128,0 (128,5)	128,0 (128,5)	25,3 (25,7)	128,1 (128,5)
C-14	127,6 (128,3)	128,0 (128,5)	128,0 (128,5)	127,3 (128,0)	25,4 (25,7)
C-15	128,0 (128,8)	25,4 (25,6)	25,5 (25,6)	130,0 (130,6)	127,0 (126,5)
C-16	25,2 (25,7)	127,6 (128,3)	127,5 (128,3)	26,9 (27,3)	131,8 (138,6)
C-17	126,8 (126,5)	128,2 (128,8)	128,2 (128,8)	29,1 (29,6)	20,4 (20,3)
C-18	131,6 (138,6)	25,3 (25,7)	25,5 (25,7)	31,2 (31,8)	14,0 (14,2)
C-19	20,2 (20,3)	126,8 (126,5)	127,0 (126,5)	22,3 (22,8)	
C-20	13,9 (14,2)	131,6 (138,6)	131,9 (138,6)	13,7 (14,2)	
C-21		20,3 (20,3)	20,4 (20,3)		
C-22		13,9 (14,2)	14,1 (14,2)		

*The values calculated by the additive scheme (see text) are given in parentheses.

Acids (I)-(III) were identified by PMR and ^{13}C NMR spectroscopy. In the assignment of the protons of the methylene groups, in addition to the multiplet structure of their resonance signals the appropriate correlation tables of chemical shifts [20] were taken into consideration. Where necessary, double proton-proton resonance was used. The features of the PMR spectra obtained (see the Experimental part), including the distribution of the integral intensity between the olefinic and methylene protons, and also between the individual methylene groups, permitted the number of double bond in the compounds studied to be determined and confirmed the methylene-separated position of these bonds and the total lengths of the chains of the PUFAs.



IV

V

The assignment of the resonance signals of the carbon atoms was made by comparing the spectra with one another and also with the spectra of standard arachidonic (IV) and α-linolenic (V) acids that we had obtained. In addition to this, in the assignment we took into consideration the chemical shifts calculated by the additive scheme proposed for Z-PUFAs by Batchelor et al. [21]. The corresponding figures are given in Table 2.

To determine the positions of the double bonds in completely methylene-separated PUFAs it is sufficient to know the position of the first and the last double bonds. The signal of the ω-1 carbon atom in each of the spectra of compounds (I)-(III) and (V) was present in the ∿20 ppm region, while in the spectrum of (IV) this signal had a shift of 22 ppm. The results of a comparison permitted the conclusion that the last double bond in each of compounds (I)-(III) was present in the ω-3 position and had the Z-configuration. The observed screening of the ω-1 carbon atom in each of compounds (I-III) and (V) by ∿2 ppm as compared with (IV) can easily be explained by the existence of a steric interaction of cis-located methylene groups in the ω-1 and ω-4 positions, leading to the appearance of a γ-effect [22-25].

Apparently, the fact that the C-3 carbon atom is screened more strongly (∿2 ppm) in compound (III) than in (I, II, IV, and V) must also be explained by the influence of the γ-effect, and this, in its turn, indicates the Z-configuration of the first double bond in this compound. On the other hand, the conclusion that all the double bonds in the acids studied have the Z-configuration can be made on the basis of the fact that the chemical shifts of the carbon atoms of the methylene groups present between the double bonds in compounds (I-III) practically coincide with the same shifts in compounds (IV) and (V). It is possible to arrive at the same conclusion on the basis of the fact that for the overwhelming number of carbon atoms the values of the chemical shifts calculated from the additive scheme with the increments proposed for the PUFAs with the Z-configuration of all the double bonds [21] are very close to the experimental values. Thus, the results obtained indicate the retention of the native structure on the use of the proposed method of isolation, which is of practical value since only Z-acids are capable of being converted into prostaglandins in the process of biosynthesis.

The purity of the acids isolated and their amounts in the lipid waste were determined by the GLC method (see Table 1). The presence in the samples analyzed of palmitic, stearic, palmitoleic, oleic, linoleic, α-linolenic, and arachidonic acids was shown by the method of adding the standard acids, and the presence of eicosenoic, eicosatetraenoic, and docosatetraenoic acids was shown by the change in the equivalent chain length (ECL) [26]. As can be seen from Table 1, an increase in the chain length of an HFA by two methylene groupings leads to a rise in the ECL by two units, while the appearance of an additional double bond increases the ECL by 0.9 units for mono- and dienoic acids and by 1.25 units in the case of polyenoic acids.

The amount of PUFAs of the α-linolenic series in the lipid waste that we investigated is small, but, in view of the considerable volumes of waste, the isolation of compounds (I-III) from it appears promising. This gives a solution to the problem of the complex processing of the raw material.

EXPERIMENTAL

The TLC of the samples investigated was performed on Silufol UV 254 (Czechoslovakia), and also on Silufol treated with a 3% solution of silver nitrate, using the following systems: 1) ether—hexane (1:1), and 2) ether—hexane—ethanol—acetic acid (75:15:2:0.1). The substances were detected on chromatograms by treating the latter with a 10% alcoholic solution of molybdophosphoric acid followed by heating the plates to 100-120°C.

The gas-chromatographic analysis of the fatty acids was performed on a Packard model 419 chromatograph with a flame-ionization detector and a Shimadzu C-RIB integrator using steel columns, 0.4 × 200 cm, filled with 10% of SP-216-PS on Supelcoport 100-120. The rate of flow of carrier gas (nitrogen) was 30 ml/min. Analysis was performed in the temperature interval of 140-200°C with a gradient of 5°C/min, the temperature of the detector and evaporator being 250°C.

For compounds (I), (II), and (IV), PMR spectra were recorded on a Bruker WM-250 spectrometer with a working frequency of 250 MHz.

The PMR spectrum of compound (III) and also the ^{13}C NMR spectra of all the acids investigated were obtained on a Bruker WP-80 spectrometer with working frequencies of 80 and 20.115 MHz, respectively. The resonance conditions were stabilized on the signal of the deuterium of the solvent. The samples for analysis were solutions of the compounds in $CDCl_3$ with concentrations of 0.30 M for (III), 1.3 M for (V), and 3.5 M for the other acids. The internal standard was $CDCl_3$, the chemical shift of which in the δ scale was taken as 7.25 ppm in the PMR spectra and 76.9 ppm in the ^{13}C NMR spectra.

Preparation of PUFAs of the α-Linolenic Series. To 1 kg of the lipid wastes from the production of insulin was added 2 liters of toluene, and the mixture was stirred for 30 min. After standing, the organic layer was separated off and the solvent was evaporated to give 200 g of a residue containing a mixture of HFAs, phospholipids, cholesterols, and other non-acid impurities. The residue was dissolved in 400 ml of toluene and the solution was deposited on a column containing 400 g of silica gel. The HFAs (R_f 0.73) [1] were eluted from the column with 3 liters of toluene, and the solvent was evaporated off to give 175 g of a concentrate of HFAs containing a total of 2.4% of (I), (II), and (III).

The HFA concentrate was dissolved in 250 ml of toluene and the solution was deposited on a column containing 880 g of silica gel and 90 g of silver nitrate. The products were eluted from the column with mixtures of toluene and ethyl acetate in the following ratios: 96:4 – 1.3 liters; 90:10 – 4.0 liters; 88:12 – 0.7 liters; 86:14 – 0.7 liters; 84:16 – 0.7 liters; 82:18 – 0.3 liters; 80:20 – 0.3 liters; and 60:40 – 2.0 liters. The following were obtained in succession: a mixture of HFAs without PUFAs of the α-linolenic series; the acid (IV); a mixture of (IV) and (II); the acid (II); a mixture of (II) and (I); the acid (I); a mixture of (I) and (III) and the acid (III). After elimination of the solvent, the following were obtained: acid (I) with a purity of 97%; 1.1 g: R_f 0.23 (2), n_D^{20} 1.4968, d_{20}^4 0.9284; PMR spectrum, δ, ppm: 0.95 t (3H, CH_3), 1.72 tt (2H, $CH_2-C-COO^-$), 2.10 m (4H, $CH_2-C=$), 2.35 t (2H, CH_2COO^-), 2.85 m (8H, $=C-CH_2-C=$), 5.35 m (10H, $-CH=CH-$), 11.52 br.s (1H, COOH); ^3J (H, H), Hz: $J(CH_3, CH_2)=J(C^3H_2, C^2H_2)=J(C^3H_2, C^4H_2) = 7$.

Acid (II) with a unity of 94%, 0.9 g: R_f 0.57 (2), n_D^{20} 1.4928, d_{20}^4 0.9218; PMR spectrum, δ, ppm: 0.98 t (3H, CH_3), 1.40 m (4H, $-CH_2-$), 1.63 m (2H, $CH_2-C-COO^-$), 2.10 m (4H, $CH_2-C=$), 2.35 t (2H, CH_2COO^-), 2.83 m (8H, $=C-CH_2-C=$), 5.37 m (10H, $-CH=CH-$), 11.78 br. s (1H, COOH); ^3J(H, H), Hz: $J(CH_3, CH_2)=J(C^2H_2, C^3H_2) = 7$;

Acid (III) with a purity of 96%, 0.25 g; R_f 0.10 (2), n_D^{20} 1.5043, PMR spectrum, δ, ppm: 0.96 t (3H, CH_3), 2.06 m (2H, $CH_2-C=$), 2.40 m (4H, $=C-CH_2-CH_2-COO^-$), 2.87 m (10H, $=C-CH_2-C=$), 5.37 m (12H, CH=CH), 10.40 br.s (1H, COOH); ^3J(H, H), Hz: $J(CH_3, CH_2) = 7$;

Acid (IV) with a purity of 96%, 6.1 g: R_f 0.89 (2), n_D^{20} 1.4850, d_4^{20} 0.9240; PMR spectrum, δ, ppm: 0.92 t (3H, CH_3), 1.32 m (6H, $-CH_2-$), 1.73 tt (2H, $CH_2-C-COO^-$), 2.10 m (4H, $CH_2-C=$), 2.35 t (2H, CH_2-COO^-), 2.82 m (6H, $=CH-CH_2-C=$), 5.40 m (8H, $-CH=CH-$), 11.81 br.s (1H, COOH); ^3J(H, H), Hz: $J(CH_3, CH_2)=J(C^3H_2, C^2H_2)=J(C^3H_2, C^4H_2) = 7$.

SUMMARY

1. Eicosa-5Z,8Z,11Z,14Z,17Z-pentaenoic, docosa-7Z,10Z,13Z,16Z,19Z-pentaenoic, and docasa-4Z,7Z,10Z,13Z,16Z,19Z-hexaenoic acids have been detected in the lipid waste from the production of insulin and their quantitative compositions has been studied.

2. A method has been developed for isolating the above-mentioned acids from this waste which differs from those described in the literature.

3. The compounds studied were identified and the positions and configurations of the double bonds were confirmed by PMR and ^{13}C NMR.

LITERATURE CITED

1. D. F. Reingold and P. Needleman, Trends Pharm., 1, 356 (1980).
2. S. M. F. Lai and W. P. Manley, Nat. Prod. Rept., 1, 409 (1985).
3. M. Hasegava, New Food Ind., 26, 26 (1985).
4. M. Yamashita and I. Kadoma, Japanese Patent Application No. 58-18317 (1983).
5. S. Tsunehara, A. Kanazawa, S. Teshima, and B. Kagihara, Japanese Patent Application No. 55-92316 (1980).
6. S. Inami, I. Kobatake, and F. Hirahara, Japanese Patent Application No. 57-31614 (1982).

7. T. Fujita and M. Makuta, Japanese Patent Application No. 57-187397 (1982).
8. S. Takayama, Japanese Patent Application No. 59-46225 (1985).
9. H. O. Bang, J. Dyerberg, J. R. Vane, and S. E. Moncada, Australian Patent No. 2033845 (1980).
10. I. Kokubu, S. Hayashi, and K. Kodama, Japanese Patent Application No. 59-59644 (1984).
11. I. Kokubu, S. Hayashi, and K. Kodama, Japanese Patent Application No. 59-67241 (1984).
12. J. Dyerberg, H. O. Bang, E. Stoffersen, S. Moncada, and F. R. Vane, Lancet, No. 2, 117 (1978).
13. T. Fujita, M. Makuta, and S. Kondo, Japanese Patent Application No. 57-169416 (1982).
14. N. Iritani, K. Inoguchi, M. Endo, E. Fukuda, and M. Mortia, Biochim. Biophys. Acta., 618, 378 (1980).
15. W. Wagu, S. Hayashi, and K. Kodama, US Patent No. 4438106 (1984).
16. M. E. Stansby, J. Am. Oil Chem. Soc., 56, 793A (1979).
17. V. E. Vas'kovskii and N. A. Romashina, Bioorgan. Khimiya, 9, 266 (1983).
18. J. Krzynowek, Food Technol., 39, 61 (1985).
19. L. A. Yakusheva, G. I. Myagkova, I. K. Sapycheva, and R. P. Evstigneeva, Khim. Prir. Soedin., 233 (1984).
20. A. J. Gordon and R. A. Ford, The Chemist's Companion, Wiley-Interscience, New York (1972).
21. J. G. Batchelor, R. J. Cushley, and J. H. Prestegard, J. Org. Chem., 39, 1698 (1974).
22. D. M. Grant and B. Cheney, J. Am. Chem. Soc., 89, 5315 (1967).
23. B. V. Cheney and D. M. Grant, J. Am. Chem. Soc., 89, 5319 (1967).
24. E. R. Prokf'ev and Zh. A. Krasnaya, Izv. Akad. Nauk SSSR, Ser. Khim., 1011 (1980).
25. I. N. Gracheva, E. P. Prokof'ev, I. R. Kovel'myan, and A. I. Tochilkii, Khimiya Geterotsikya. Soedin., 1065 (1985).
26. G. R. Khan and F. Scheinmann, Prog. Chem. Fats Other Lipids, 15, 343 (1978).

RESIN ACIDS OF THE OLEORESINS OF CONIFERS GROWING IN TRANSCARPATHIA

V. I. Bol'shakova, L. I. Demenkova, É. N. Schmidt, and V. A. Pentegova

UDC 547.914.2

The chemical composition of the resin acids of the oleoresins of five species of the conifers of Transcarpathia has been studied. The oleoresins contain eight resin acids. $\Delta^{8,9}$-Isopimaric and anticopalic acids have been found in the acid part of the oleoresin of the Weymouth pine.

Continuing a systematic investigation of oleoresins of conifers of the USSR [1], we have studied the compositions of the resin acids of several species having small areas in Transcarpathia with a chemical composition of which there is no information in the literature.

In the present communication we give the results of a study of the acid fractions of the oleoresins of *Abies alba* Mill. (silver fir), *Larix decidua* Mill. (European larch), *Pinus mugo* T. (Swiss mountain pine), *Pinus strobus* Ladb. (Weymouth pine), and *Picea excelsa* L.* and *Picea abies* K. (Norway spruce†), growing in Transcarpathia. The Swiss stone pine, having a limited area in Transcarpathia, belongs to the peculiar species series *Montanae* [2].

*The oleoresin of the Norway spruce *Picea excelsa* was collected in Chelyabinsk province.
†The Russian text gives for *P. excelsa* L. a name translating as "common spruce" and for *P. abies* K. "European spruce." According to all the reference works consulted the Latin names are synonymous and apply to the Norway spruce [Translator].

Novosibirsk Institute of Organic Chemistry, Siberian Branch, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 212-214, March-April, 1987. Original article submitted July 30, 1986.

0009-3130/87/2302-0173$12.50