CHEMICAL INVESTIGATION OF *Schizandra chinensis*

COMMUNICATION 4. ISOLATION, STRUCTURE, AND SYNTHESIS OF DEOXYSCHIZANDRIN, AND THE STRUCTURE OF γ-SCHIZANDRIN

(UDC 615.785)

N. K. Kochetkov, A. Ya. Khorlin, and O. S. Chizhov

Institute for the Chemistry of Natural Products, Academy of Sciences, USSR
Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 6, pp. 1036-1042, June, 1964
Original article submitted November 20, 1962

As reported previously [1], in the chromatographic separation of the unhydrolyzable part of the seed oil from Schizandra chinensis we found, together with the main component schizandrin [2], a considerable amount of closely related substances. Our attention was turned in the first place to the separation of the more polar fractions of this complex mixture, of which we isolated schizandrin and schizandrol. As regards the less polar substances contained in Fractions 3 and 4 from the first rough fractionation of the oil [1], we isolated only one optically inactive substance in the pure state—γ-schizandrin, m.p. 92-93°. We have now succeeded in isolating from these fractions two more substances related to schizandrin which are present in the schizandra oil in small amounts. This paper reports the isolation and examination of the structure of these substances and also of γ-schizandrin, which is of interest in relation to the study of a new group of natural compounds—5,6,7,8-tetrahydrodibenzo[a, c]cyclooctene derivatives [2]. After the crystallization of γ-schizandrin from a solution of Fractions 3 and 4 the mother solution was chromatographed on alumina. From fractions eluted with benzene and 95 : 5 benzene-chloroform we isolated a substance that was isomeric with γ-schizandrin and was extremely close to it in all of its properties with the exception of specific rotation ($[\alpha]^{20}_D$ −87°). This substance was named pseudo-γ-schizandrin. From the fractions obtained by the elution of the column with 1 : 1 benzene-chloroform we isolated yet another pure substance, m.p. 116° and $[\alpha]^{20}_D$ + 107°, which we named deoxyschizandrin, whose structure we were able to establish and to confirm by total synthesis. According to analysis deoxyschizandrin has the molecular formula $C_{24}H_{32}O_6$ and contains six methoxy groups. We were able to come to a fairly firm conclusion on the structure of deoxyschizandrin from the ultraviolet, infrared, and NMR spectra. The ultraviolet spectrum of deoxyschizandrin is very characteristic (Fig. 1) and is almost identical with that of schizandrin, which clearly indicates that the new substance is a derivative of 5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxydibenzo[a, c]cyclooctene[2-4]. The infrared spectrum (Fig. 2) shows the presence of an aromatic system in deoxyschizandrin and the absence of any functional groups, including hydroxy groups, which are present in schizandrin [1, 2]. The NMR spectrum of deoxyschizandrin (Fig. 3) contains peaks at 18 Hz (two aromatic protons), 123 and 135 Hz (methoxy groups), 177 and 192 Hz (protons of methylene groups in the α-position to the aromatic nucleus), and 236, 242, 247, and 253 Hz (two CH_3—CH groups); there are no peaks corresponding to any other groups. The ultraviolet, infrared, and NMR data cited correspond to the structure 5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[a, c] cycloocetne (I), which must now be assumed for deoxyschizandrin.

Investigation showed that the structure of γ-schizandrin, which was isolated earlier [1], can also be established on the basis of available data relating its structure to that of deoxyschizandrin. Verification of previous analytical data [1] confirmed that γ-schizandrin has the molecular formula $C_{23}H_{28}O_6$, but that γ-schizandrin actually contains four methoxy groups, and not three as indicated by the earlier analytical results [1]. The sources of such errors in the determination of methoxy groups in schizandrin has already been discussed by us [3]. Since the ultraviolet spectrum of γ-schizandrin is almost identical with that of schizandrin [1], it must be assumed that γ-schizandrin is also a 5,6,7,8-tetrahydrodibenzo[a, c]cyclooctene derivative [2-4]. The infrared spectrum indicates the presence of an aromatic system and the absence of hydroxy and other functional groups. The NMR spectrum of γ-schizandrin (Fig. 4) indicates the presence of two nonequivalent but very close chemical shifts of aromatic protons (peaks at 15.8 and 19.2 Hz) and of peaks due to the methylenedioxy groups (41 Hz), four methoxy groups (125 and 138 Hz; ratio of areas

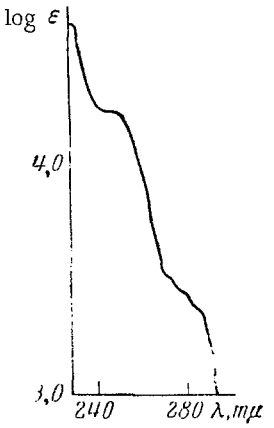


Fig. 1. Ultraviolet spectrum of deoxyschizandrin.

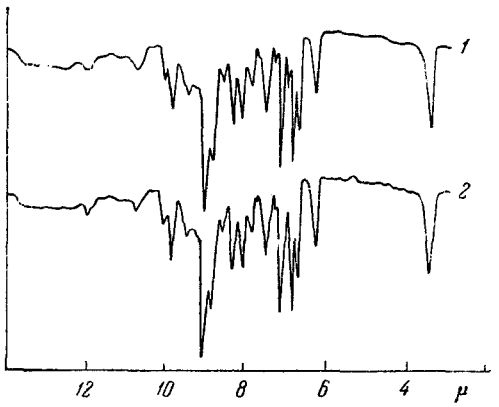


Fig. 2. Infrared spectrum of natural (+)-deoxyschizandrin (1) and of synthetic racemic deoxyschizandrin (2).

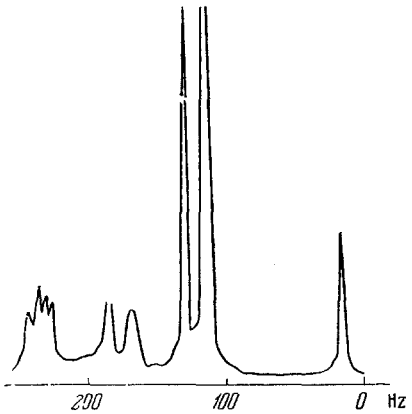


Fig. 3. NMR spectrum of deoxyschizandrin.

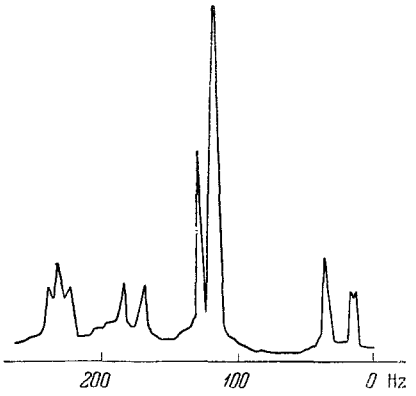
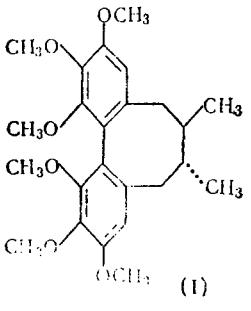
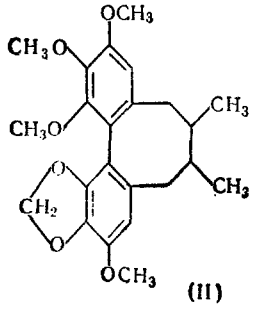


Fig. 4. NMR spectrum of γ-schizandrin.

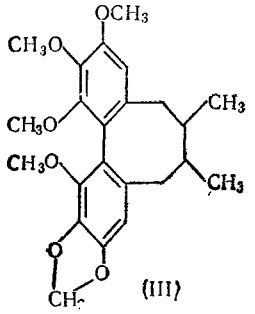
3 : 1), two methylene groups in the α-position to aromatic nuclei (172 and 190 Hz), and two methyl groups (238, 245, and 251 Hz). It follows from these data that the structure of γ-schizandrin may be represented by (II) or (III).



(I)



(II)

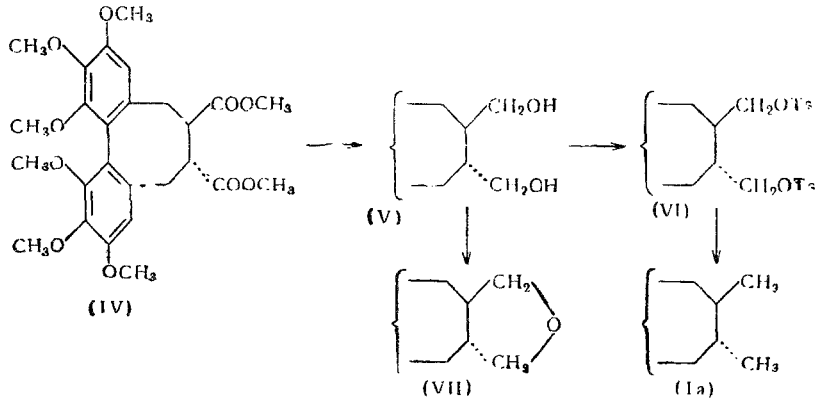


(III)

It is known [5] that the chemical shift of methoxy groups so positioned that the magnetic field of a neighboring aromatic nucleus acts on them is greater than the average value of the chemical shift of methoxy groups. Incidently, it is just this that may explain the formation of two peaks belonging to methoxy groups in the NMR spectra of schizandrin [2, 3] and deoxyschizandrin, and of these the smaller peak, which is shifted more toward the stronger fields, must belong to methoxy groups in the 1- and 12-positions. Comparison of the areas of the peaks at 125 and 138 Hz in the NMR spectrum of γ-schizandrin shows that only one methoxy group is under the action of the magnetic field of a neighboring aromatic nucleus, whereas the other three give the usual chemical shift for aromatic methoxyls. For γ-schizandrin, therefore, the formula (II) seems more probable than the formula (III), for in the latter case one would

expect the appearance of two equal peaks in the 120-140 Hz region. To confirm the proposed structure of γ-schizandrin it was necessary to convert it into the corresponding hexamethoxy derivative, identical or isomeric to deoxyschizandrin. This could be achieved by the cleavage of the methylenedioxy group with subsequent methylation of the dihydric phenol obtained and was not an easy task in view of the lack of simple methods for the selective removal of a methylenedioxy group and the extremely ready oxidizability of polymethoxy dihydric phenols. Three methods have been described for the elimination of the methylenedioxy group: hydrolysis with acids in presence of phloroglucinol under severe conditions [6]; action of phosphorus pentachloride with subsequent hydrolysis of the reaction product with aqueous sodium carbonate solution and methanolysis of the then formed cyclic carbonate [7]; and heating with a concentrated solution of sodium methoxide or potassium hydroxide in methanol at 170-180° [8]. When we applied the first method to γ-schizandrin we were unable to separate the phenolic compound formed from the phloroglucinol-formaldehyde polymer. When treated with phosphorus pentachloride and subsequent methylation γ-schizandrin gave a complex mixture of substances; the yield of a substance corresponding approximately in R_f value to deoxyschizandrin was negligible, and we were unable to obtain it in the pure state. Only when we used the third method—treatment with sodium methoxide and subsequent methylation of the phenol formed with dimethyl sulfate—did we obtained a crystalline substance $C_{24}H_{32}O_6$, corresponding in composition to natural deoxyschizandrin, but differing sharply from it in specific rotation; we named it isodeoxyschizandrin. The melting points, R_f values in various solvent systems, and infrared spectra of natural deoxyschizandrin and of isodeoxyschizandrin were found to be very close, from which it may be supposed that the two compounds have the same structure and differ only in their stereochemistry.

For final confirmation of the structure of deoxyschizandrin as 5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[a, c]cyclooctene we carried out the total synthesis of the racemate of this compound, which is the first total synthesis of a natural 5,6,7,8-tetrahydrodibenzo[a, c] cyclooctene. The scheme of the synthesis was as follows:



The dimethyl ester (IV), whose synthesis we have described earlier [4], was reduced into the corresponding trans diol (V) with lithium aluminum hydride in tetrahydrofuran. Without further purification the glassy diol (V) was converted into the di-p-toluenesulfonate (VI) by means of p-toluenesulfonyl chloride in pyridine at +5°. For the preparation of (VI) it is essential to use a large excess of p-toluenesulfonyl chloride and to take care that the reaction temperature does not rise above the level indicated, otherwise a considerable amount of the anhydro compound (VII) is formed (cf. [9]). The di-p-toluenesulfonate (VI) was reduced with lithium aluminum hydride to racemic deoxyschizandrin (Ia). Natural and synthetic deoxyschizandrins have the same R_f values in thin-layer chromatography on alumina. Their infrared spectra, determined in carbon tetrachloride solution, are completely identical (Fig. 2). Comparison of the properties of the compounds obtained in conjunction with some data from the literature makes it possible to arrive at some fairly definite views on their stereochemistry.

A compound with the structure of 5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[a, c]-cyclooctene, which has two asymmetric centers, may exist as the following stereoisomers: the (+)- and (−)-trans-isomers, their racemate, and the cis isomer, which is a meso form, for it is known [10] that, when separated into their enantiomers, symmetrical cis-5,6,7,8-tetrahydrodibenzo[a, c] cyclooctenes readily racemize again because of rotation around the aryl-aryl bond. It is known also [11] that such relatively small substituents as methoxy groups in the 1- and 12-positions inhibit such rotation only to a slight extent. On the other hand, it has been shown that trans-6,7-disubstituted 5,6,7,8-tetrahydrodibenzo[a, c]cyclooctenes cannot racemize [10]. In spite of the fact that in the isolation of deoxyschizandrin the original mother solutions were kept for 18 months and were heated several times in the

Chromatography of Fractions 3 and 4 [1] on Alumina

Fraction No.	Solvent	Vol., liters	Residue g	Composition according to thin-layer chromatography*
3, 4a	Petroleum ether	1	0.6	α-Schizandrin
3,4b	Pet. ether + benzene 95 : 5	1	0.7	α-Schizandrin, β-schizandrin
3,4c	Ditto 9 : 1	1	0.6	β-Schizandrin**
3,4d	" 4 : 1	1	0.5	β- and γ-Schizandrins
3,4e	" 1 : 1	1	0.7	β- and γ-Schizandrins
3,4f	Benzene	2	4.2	γ-Schizandrin
3,4g	Ditto 95 : 5	2	2.1	γ-Schizandrin***
3,4h	" 9 : 1	2	2.2	γ-Schizandrin, deoxyschizandrin
3,4i	" 1 : 1	2	1.4	Deoxyschizandrin

*R_f values: α-Schizandrin 0.64, β-schizandrin 0.55, γ-schizandrin 0.49, deoxyschizandrin 0.40.

** Attempts to prepare crystalline α-schizandrin from Fraction 3,4a and crystalline β-schizandrin from Fraction 3,4c by crystallization from petroleum ether, methanol, or ether at −60° were not successful.

***Since γ- and pseudo-γ-schizandrins have identical R_f values, the fact that the spot with R_f 0.49 belongs to a substance differing from γ-schizandrin was discovered only later.

course of treatment, the deoxyschizandrin isolated from them had a high specific rotation. From this it follows that deoxyschizandrin is one of the optically active trans isomers. It is clear that the 5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[a, c]cyclooctene obtained synthetically was, in accordance with the method of preparation used, a racemate of the trans compound. Moreover, since isodeoxyschizandrin prepared from γ-schizandrin differs in its constants both from natural and from synthetic deoxyschizandrin, it is evidently the cis isomer. Hence, in γ-schizandrin also the 6- and 7-methyl groups are cis relative to one another. The absence of optical activity in γ-schizandrin, which is rather unexpected, is probably to be explained on the view that although the γ-schizandrin molecule is not completely symmetrical, and consequently its complete racemization as a result of rotation around the aryl-aryl bond is impossible for compounds with a cis configuration at the 6- and 7-carbon atoms, an extremely low specific rotation would be expected. The structure of pseudo-γ-schizandrin has not yet been proved. As stated above, this compound has a molecular formula identical to that of γ-schizandrin. The two substances have identical R_f values in thin-layer chromatography on alumina, identical melting points, and their mixtures melt without depression. Their infrared spectra are very close, but the substances differ in specific rotation. On the basis of these data it may be supposed that pseudo-γ-schizandrin is the trans isomer of γ-schizandrin.

EXPERIMENTAL

Ultraviolet spectra were determined with an SF-4 spectrophotometer in alcoholic solutions, infrared spectra were determined with an IKS-14 instrument in carbon tetrachloride solutions, and NMR spectra were determined with a JMN-3 instrument at 40 MHz with benzene as external standard in chloroform solution for deoxyschizandrin and in carbon tetrachloride solution for γ-schizandrin. For preparative chromatography we used alumina of Brockmann activity II. As solvent for thin-layer chromatography we used a 1 : 4 mixture of ethyl acetate and heptane. All values of $[\alpha]_D$ were measured in chloroform. Values of melting point are not corrected.

γ-Schizandrin, which crystallized out from Fractions 3 and 4 [1], was purified by recrystallization from methanol and heptane; m.p. 93-95° $[\alpha]_D^{20}$ 0 ±1° (c 3,0). Found: OCH_3 31.07; 30.80%. $C_{23}H_{28}O_6$. Calculated: 4 OCH_3 30.97%.

Isolation of Deoxyschizandrin and Pseudo-γ-schizandrin. The mother solution from the crystallization from γ-schizandrin was evaporated, and the residue (14.5 g) was chromatographed on 600 g of alumina. The chromatography results are presented in the table.

Fractions 3,4f and 3,4g were dissolved in 30 ml of hexane. After a few days 2.8 g of pseudo-γ-schizandrin crystallized out; m.p. 93-95° (after five crystallizations from methanol); $[\alpha]_D^{20}$ −87° (c 2.8). A mixture with γ-schizandrin melted without depression. Found: C 68.79; 68.87; H 6.99; 6.95; OCH_3 31.04, 30.97%. $C_{23}H_{28}O_6$. Calculated: C 68.98; H 7.04; 4 OCH_3 30.97%.

Fraction 3,4i was dissolved in 5 ml of ether, and 25 ml of hexane was added. After a few hours deoxyschizandrin started to crystallize out; we obtained 630 mg of product, m.p. 116-117° (from methanol), $[\alpha]_D^{20}$ + 107° (c 5.7). Ultraviolet spectrum (see Fig. 1): λ_{max} 248 mμ (log ε 4.218); λ_{infl} 273 mμ (log ε 3.490) and 283 mμ (log ε 3.380). NMR spectrum, see Fig. 3. Infrared spectrum, see Fig. 2. Found: C 69.05; 69.11; H 7.66; 7.54; OCH_3 44.72; 44.76%. $C_{24}H_{32}O_6$. Calculated: C 69.20; H 7.75; OCH_3 44.70%.

Isodeoxyschizandrin. 1.0 g of γ-schizandrin was heated in a sealed tube with sodium methoxide solution, prepared from 1.5 g of sodium and 20 ml of methanol, for ten hours at 175-180°. The tube was opened, and methanol was vacuum-distilled off in a stream of nitrogen: 10 ml of water was added, and neutral substances were extracted with ether. 7 ml of dimethyl sulfate was added to the aqueous alkaline solution, and the reaction mixture was stirred until it became acid. 10 ml of 25% sodium hydroxide solution and a further 5 ml of dimethyl sulfate were then added, and the reaction mixture was stirred until the spontaneous evolution of heat stopped, after which a further 5 ml of 25% sodium hydroxide solution was added, and the reaction mixture was heated in a water bath for 30 minutes. The mixture was then cooled, the oil that separated was extracted with ether, the ethereal solution was washed with water and dried with sodium sulfate, and ether was driven off. The oily residue was dissolved in 5 ml of methanol, the solution was placed in a tube, 20 ml of 25% methanolic potassium hydroxide was added, and the tube was sealed and heated for ten hours at 175-180°, after which the reaction products were methylated with dimethyl sulfate as described above. This time the oil remaining after the removal of ether partially crystallized on standing. The crystals were washed with a little methanol and recrystallized from the same solvent. Yield 91 mg; m.p. 116-117° $[\alpha]_D^{20}$ 0 ±1° (c 2.5). Found: C 69.40; 69.30; H 7.70; 7.76%. $C_{24}H_{32}O_6$. Calculated: C 69.20; H 7.75%.

(±)-Deoxyschizandrin. 1 g of lithium aluminum hydride in 15 ml of dry tetrahydrofuran was added to 1.2 g of the dimethyl ester (IV) in 25 ml of dry tetrahydrofuran. The mixture was stirred for 30 minutes at room temperature and then for 90 minutes at 50-60°. The reaction mixture was then cooled to 0°, and the excess of lithium aluminum hydride was decomposed with 15 ml of ethyl acetate; 100 ml of ether, 20 ml of water, and 20 ml of 10% hydrochloric acid were added. The ether layer was separated, and the aqueous layer was extracted with three 50-ml portions of ether; the combined ether extracts were washed with potassium bicarbonate solution and water and were dried with sodium sulfate; ether was driven off. The glassy diol (V) obtained (1 g) was dissolved in 10 ml of dry pyridine, the solution was cooled to 0°, and 3 g of solid p-toluenesulfonyl chloride was added. The mixture was left at 0° for four hours, poured onto ice, and left overnight. The precipitated p-toluenesulfonate (VI) was extracted with chloroform, the extract was washed with 10% sulfuric acid, with potassium bicarbonate solution, and with water, and chloroform was driven off. The residue was dissolved in 50 ml of dry tetrahydrofuran, 1.5 g of lithium aluminum hydride was added, and the mixture was stirred for 30 minutes at room temperature, then for two hours at 40-50°, and finally for one hour at the boil. When the mixture had cooled excess of lithium aluminum hydride was decomposed with 15 ml of ethyl acetate, and then 15 ml of water, 100 ml of ether, and 50 ml of 10% hydrochloric acid were added. The ether layer was separated, the aqueous layer was extracted with three 50-ml portions of ether, and the combined ether extracts were washed with potassium hydroxide solution and water. Ether was driven off, and the residue (700 mg) was chromatographed on 20 g of alumina, with elution with benzene and the collection of 25-ml fractions. The whole of the deoxyschizandrin was eluted in the first six fractions. Yield 500 mg; m.p. 131-132° (from methanol). Like natural deoxyschizandrin, the substance had R_f 0.40. The two substances had identical infrared spectra (see Fig. 2). Found: C 69.22; 69.39; H 7.79; 7.93%. $C_{24}H_{32}O_6$. Calculated: C 69.20; H 7.75%.

SUMMARY

1. From the unhydrolyzable part of the seed oil of Schizandra chinensis two new substances were isolated: deoxyschizandrin and pseudo-γ-schizandrin.

2. The structures of deoxyschizandrin and γ-schizandrin were established; they belong to a new group of natural substances derived from 5,6,7,8-tetrahydrodibenzo[a, c]cyclooctene. The structure of deoxyschizandrin was confirmed by the total synthesis of its racemate.

3. The stereochemistry of deoxyschizandrin, γ-schizandrin, and pseudo-γ-schizandrin is discussed.

LITERATURE CITED

1. N. K. Kochetkov, A. Ya. Khorlin, and O. S. Chizhov, Zh. obshch. khimii 31, 3554 (1961).
2. N. K. Kochetkov, A. Ya. Khorlin, O. S. Chizhov, and V. I. Sheichenko, Tetrahedron Letters, No. 20, 730 (1961).
3. N. K. Kochetkov, A. Ya. Khorlin, O. S. Chizhov, and V. I. Sheichenko, Izv. AN SSSR. Otd. khim. n., 1962, 850.

4. N. K. Kochetkov, A. Ya. Khorlin, and O. S. Chizhov, Izv. AN SSSR. Otd. khim. n. 1962, 856.
5. I. R. Bick, J. Harley-Mason, H. Sheppard, and J. Vernengo, J. Chem. Soc. 1961, 1896.
6. E. Späth and H. Quietensky, Ber. 60, 1882 (1927).
7. S. V. Lieberman, G. P. Mueller, and E. T. Stiller, J. Amer. Chem. Soc. 69, 1540 (1947).
8. T. Kaku and H. Ri, J. Pharm. Soc. Japan 57, 289 (1937); S. Keimatsu and T. Ishiguro, J. Pharm. Soc. Japan, 56, 61 (1936); G. K. Hughes and E. Ritchie, Austral. J. Chem. 7, 104 (1954); A. J. Birch, G. K. Hughes, and E. Smith, Austral. J. Chem. 7, 83 (1954).
9. A. W. Schrecker and J. L. Hartwell, J. Amer. Chem. Soc. 77, 432 (1955).
10. L. V. Dvorken, R. B. Smith, and K. Mislow, J. Amer. Chem. Soc. 80, 486 (1958).
11. L. A. Wiles, Chem. Rev. 56, 329 (1956).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.