4. S. Imai, E. Murata, S. Fujioka, M. Koreeda, and K. Nakanishi, J. Chem. Soc., Chem. Commun., 546 (1969).
5. U. Baltaev, N. D. Abdullaev, M. R. Yagudaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 813 (1977).
6. B. Z. Usmanov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 466 (1975).
7. M. Koreeda, K. Nakanishi, S. Imai, T. Tsuchiya, and N. Wasada, Mass Spectroscopy, 17, 669 (1969).
8. J. P. Girault and R. Lafont, J. Insect Physiol., 34, 701 (1988).
9. P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mulari, and E. Wenkert, J. Am. Chem. Soc., 90, 5480 (1968).
10. A. N. Svechnikov, R. U. Umarova, M. B. Gorovits, K. L. Seitanidi, Ya. V. Rashkes, M. R. Yagudaev, and N. K. Abubakirov, Khim. Prir. Soedin., 67 (1981).
11. M. N. Galbraith and D. H. S. Horn, Austr. J. Chem., 22, 1045 (1969).
12. S. L. Patts and Y. N. Shoolery, J. Magn. Reson., 46, 535 (1982).
13. M. M. Benidze, O. V. Dzhikiya, T. A. Pkheidze, E. P. Kemertelidze, and A. S. Shashkov, Khim. Prir. Soedin., No. 4, 537-542 (1987).
14. Z. Saatov, M. B. Gorovits, N. D. Abdullaev, B. Z. Usmanov, and N. K. Abubakirov, Khim. Prir. Soedin., 738 (1981).
15. I. L. Zatsny, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 175 (1973).
16. T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, Tetrahedron Lett., 375 (1968).
17. H. Rimpler, Tetrahedron Lett., 329 (1969)
18. J. W. Blunt, A. G. Lane, H. G. M. Munro, and B. G. Russel, Austr. J. Chem., 32, 779 (1979).
19. Z. Saatov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 767 (1987).
20. J. Jizba, V. Herout, and F. Šorm, Tetrahedron Lett., 5139 (1967).
21. A. Guerriero and F. Pietra, Comp. Biochem. Physiol., 80B, 277 (1985).

CIRCULAR DICHROISM OF STEROIDS WITH A LACTONE RING B. BRASSINOSTEROIDS AND COMPOUNDS RELATED TO THEM

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The circular dichroism spectra of steroids having the lactone group in ring B that is characteristic for brassinosteroids, and their isomers, have been studied. It has been shown that in the spectra of the isomeric B-homo-7-oxa-6-keto-steroids and B-homo-6-oxa-7-ketosteroids differences are observed in the sign of the Cotton effect of the $n-\pi^*$ transition of the carboxy group, which can be used for proving their structures.

The brassinosteroids, plant growth regulators, are, chemically, $C_{27}-C_{29}$-polyhydroxy-steroids usually having in ring B a 6-keto group or a lactonic B-homo-7-oxa-6-keto group [1]. The presence of a large number of functional groups with strictly determined stereochemistry in the molecules of the brassinosteroids makes it quite unavoidable to use modern physico-chemical methods to establish their structures. These include the circular dichroism method, which has been used for proving the presence of a 6-keto group and a trans-A/B linkage in the molecules of the brassinosteroids castasterone [2] and 2-deoxycastasterone [3]. At the same time, there is no information on studies of the circular dichroism of brassinosteroids with a lactone ring B, which include, for example, 24-epibrassinolide (I). As a result of the realization of our program on the synthesis of various analogs of brassinosteroids from

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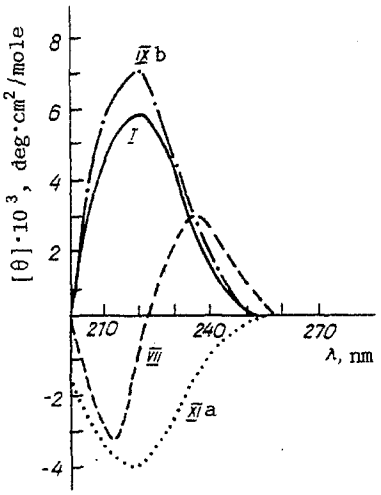


Fig. 1. Circular dichroism spectra of steroid B-lactones.

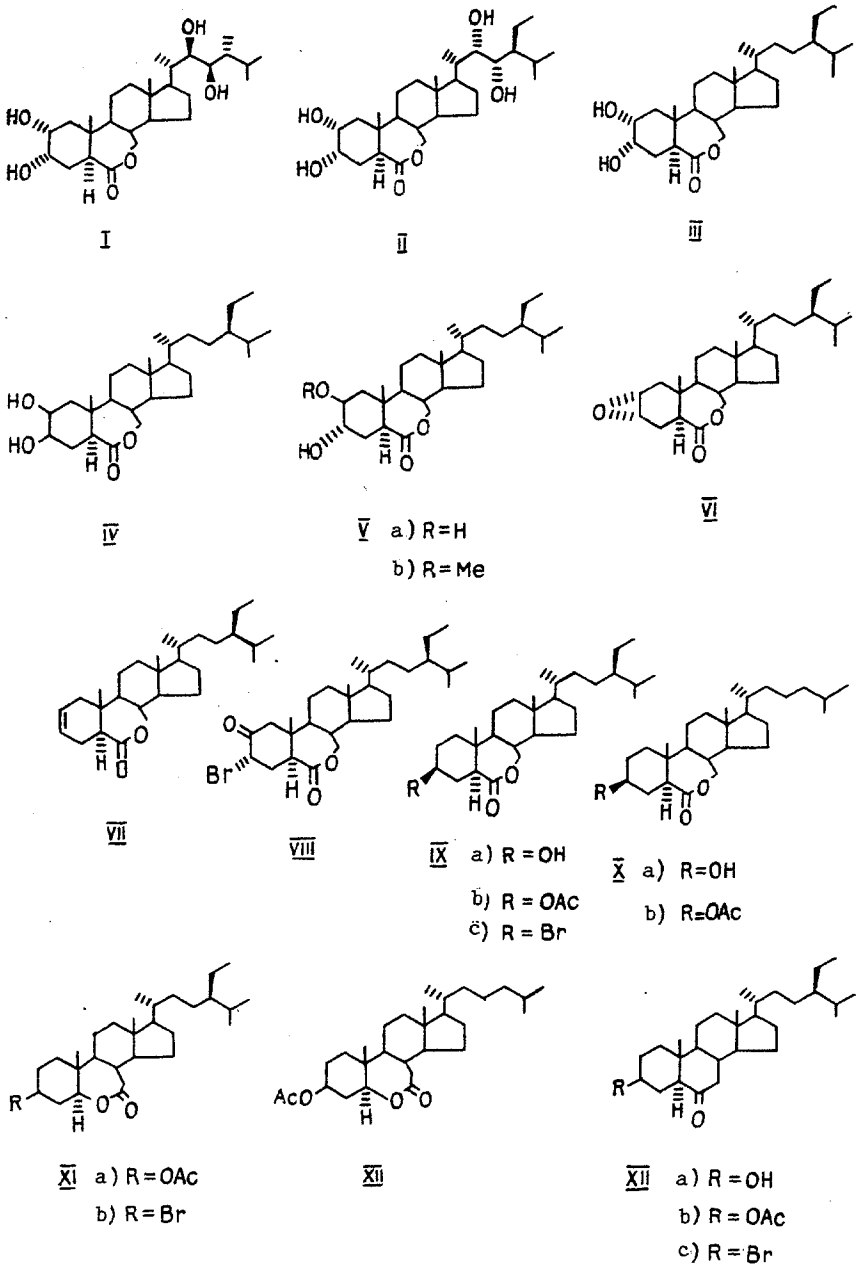
sterols, primarily β-sitosterol [4], a broad set of compounds (I-XII) having lactone groups in rings B either of the type characteristic for natural substances or of a type isomeric to it has been obtained. The two possible lactones are produced in the form of difficultly separable mixtures as the result of the oxidation of initial 6-ketosteroids of the (XIII) type by the Baeyer–Villiger reaction [4, 5]. It must be mentioned that the substances with B-lactone groups isomeric with those of the natural brassinosteroids do not possess a high activity as plant growth stimulators. Therefore, the elucidation of the precise structure of the lactones formed in the Baeyer–Villiger reaction is of great importance in the chemical synthesis of brassinosteroids.

We are the first to have studied the circular dichroism spectra of a number of brassinosteroids and compounds related to them with lactone rings B. The results obtained have been collected into a Table which also gives details of the UV spectra of the substances under discussion. The CD spectra of some typical representatives of the compounds studied are given in Fig. 1.

It must be mentioned that the UV spectra proved to be almost uninformative in the elucidation of the stereochemical features of the steroid compounds under consideration, and our main attention was devoted to a study of the CD spectra. For conformational assignments in the lactones we made use of an empirical sector rule establishing a correlation between the sign of the Cotton effect (CE) of the long-wave n–π* transition of the carboxy group and the absolute configuration of its carbon atom [6, 7]. According to this rule, the space around the lactone group is divided into sectors with the aid of planes passing through the carbon atom (Fig. 2). Then the signs of the lactone sectors must be opposite to the signs of the octants used in the octant rule for ketones [6, 7].

Starting from the octant rule, the negative sign of the CE of n–π* transition of the keto chromophores of steroids (XIIIa-c) at 292 nm (see Table 1) which is common for trans-A/B-6-ketosteroids, can be explained by the location of the bulk of the molecule in the negative upper right rear octant. As follows from a consideration of Dreiding molecular models, the atoms of rings C and D and the C-7 atom of ring B fall into this octant, while only the atoms of ring A are located in the positive upper rear octant.

On comparing the sector rule with the octant rule, for the 6-oxa-7-ketolactones (XIa, b) and (XII) one should expect a negative CE of the n–π* transition of the carboxyl, since the C-9, C-11, and C-12 atoms, and possibly, individual atoms of the side chain fall into the negative left upper sector located to the rear, while the positive right upper sector remains free. As can be seen from Table 1, for the 6-oxa-7-ketolactones a negative CE is actually observed in the 216-218 nm region. The 7-oxa-6-keto-isomers correspond to them (IXb, c) and (Xb) exhibit a positive CE of the n–π* transition, which can be explained by the contribution of the C-9 and C-11 atoms and, possibly, individual atoms of the side chain falling into the positive right upper rear sector, while only the C-4 atom is located in the negative upper left rear sector. Other 7-oxa-6-ketosteroids in addition to the brassinosteroids (VII) and (VIII) with additional C–Br and C=C chromophores also have positive CEs. It is obvious that the sign of the CE in the 7-oxa-6-keto- and 6-oxa-7-keto-isomers may serve as a convenient and fairly reliable characteristic in their identification.



Certain characteristic features can be found in a comparison of the absolute values of the molecular ellipticity [θ] of the spatial isomers. Thus, a higher value of the molecular ellipticity is observed for the cholestane 6-oxa-7-ketobrassinosteroid (XII) than for stigmastane analogs (XIa, b) while the cholestane 7-oxo- 6-ketosteroid (Xb) has a smaller ellipticity than the corresponding stigmastane 7-oxa-6-ketobrassinosteroids (IXb, c). At the same time, the sums of the absolute values of the molecular ellipticities of the corresponding isomers (IXb) and (XIa), (Xb), and (XII), and (IXc) and (XIb) remain identical, which is apparently not fortuitous.

It is known that the magnitude of the CE of a n—π* transition depends both on the number of atoms falling into the corresponding sector and on their remoteness from the lactone chromophore [7]. Attempts that we have made to link the magnitude of the CE to the number of atoms located in the various sectors have not always proved successful. Thus, the higher ellipticity of the CE of the cholestane 3β-acetoxy-6-oxa-7β-steroid (XII) than of its stigmastane isomer (XIa) is logically explained by a more effective location in the left upper sector of some of the atoms of the less branched chain. It is obvious that, for the same reason, the 3β-acetoxy-7-oxa-6-ketosteroid (Xb) should have a greater ellipticity of the CE than the isomer (IXb), but in actual fact the opposite pattern is observed.

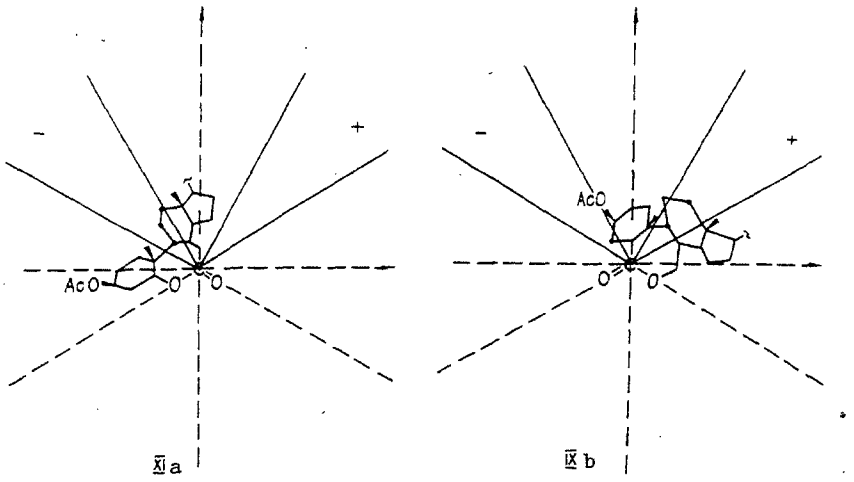


Fig. 2. Sector projections of the steroid B-lactones (XIa) and (XIb).

TABLE 1. Details of the UV and CD Spectra of Ovasteroids

Name of the compound	UV spectrum		CD spectrum	
	λ, nm	ε, liter/mole·cm	λ, nm	$[\theta]\cdot10^3$, deg·cm²/mole
B-Homo-7-oxa-6-ketosteroids				
I. 24-Epibrassinolide	220	70	220	5,8
II. (22S,23S)-28-Homobrassinolide	220	240	220	5,4
III. 22,23-Dideoxy-28-homobrassinolide	210	v.w.	220	4,0
IV. (24R)-2β,3β-Dihydroxy-B-homo-7-oxa-5α-stigmastan-6-one	210	v.w.	220	2,7
Va. (24R)-2β,3α-Dihydroxy-B-homo-7-oxa-5α-stigmastan-6-one	210	v.w.	220	3,4
Vb. (24R)-3α-Hydroxy-2β-methoxy-B-homo-7-oxa-5α-stigmastan-6-one	210	v.w.	220	2,5
VI. (24R)-2α,3α-Epoxy-B-homo-7-oxa-5α-stigmastan-6-one	206	160	225	9,5
VII. (24R)-B-Homo-7-oxa-5α-stigmast-2-en-6-one	206	670	210	−3,3
			235	3,0
VIII. (24R)-3α-Bromo-B-homo-7-oxa-5α-stigmastane-2,6-dione	208	400	208	−3,1
	312	70	310	14,0
IXa. (24R)-3β-Hydroxy-B-homo-7-oxa-5α-stigmastan-6-one	218	260	220	7,0
IXb. (24R)-3β-Acetoxy-B-homo-7-oxa-5α-stigmastan-6-one	212	150	220	7,0
IXc. (24R)-3β-Bromo-B-homo-7-oxa-5α-stigmastan-6-one	210	290	220	6,3
Xa. 3β-Hydroxy-B-homo-7-oxa-5α-cholestan-6-one	207	150	220	5,8
Xb. 3β-Acetoxy-B-homo-7-oxa-5α-cholestan-6-one	212	100	220	5,0
B-Homo-6-oxa-7-ketosteroids				
XIa. (24R)-3β-Acetoxy-B-homo-6-oxa-5α-stigmastan-7-one	212	220	218	−3,7
XIb. (24R)-3β-Bromo-B-homo-6-oxa-5α-stugnastab-7-one	213	270	218	−4,5
XII. (3β)-Acetoxy-B-homo-6-oxa-5α-cholestan-7-one	215	200	216	−5,7
6-Ketosteroids				
XIIIa. (24R)-3β-Hydroxy-5α-stigmastan-6-one	269	400	<200	±
	289	40	292	−6,2
XIIIb. (24R)-3β-Acetoxy-5α-stigmastan-6-one	210	370	<200	±
	288	35	292	−5,5
XIIIc. (24R)-3β-Bromo-5α-stigmastan-6-one	209	1200	<200	±
	288	120	293	−6

Also not fully understood are the different ellipticities of the stigmastane 6-oxa-7-ketosteroids (XIa, b), since their 3β-substituents do not fall into any of the sectors determining the sign of the CE. On the other hand, in the 7-oxa-6-ketosteroids a 3β-substituent may be located in the left lower sector and should exert an influence on the magnitude of the molecular ellipticity. However, the molecular ellipticities of the CEs of the brassinosteroids (IXa-c) and (Xa and b) considered here do not depend appreciably on the type of substituent and, moreover, even coincide for compounds (IXa and b).

The latter fact appears surprising, since the hydroxy function and the more "voluminous" acetate group in the 3β-position make the same contribution to the optical rotation. In this connection, it is interesting to compare the CDs of the 7-oxa-6-ketostigmastanes (I-Va and b),

differing by the stereochemistry of the substituents at C-2 and C-3. As can be seen from Table 1, the molecular ellipticities of the n–π* transitions of the carboxylic chromophores of these disubstituted brassionsteroids are appreciably lower than for the 3β-substituted stigmastane brassinosteroids. At the same time, the lowest ellipticity values are characteristic for compound (IV) with two β-hydroxy groups and compound (Vb) with a 2β-methoxy group. It is obvious that both the methoxy group in steroid (Vb) and the two hydroxy groups oriented correspondingly by an intramolecular hydrogen bond in compound (IV) fall into the negative left upper sector. This leads to a decrease in the positive molecular ellipticity, while the contribution of the 2β-methoxy group must be more considerable. At the same time, neither of the two α-hydroxy groups in each of compounds (I-III) falls into a left sector and they should not change the optical rotation, while in the case of the brassinosteroid (Va) with 1β-hydroxy function it is natural to expect a smaller contribution to the lowering of the molecular ellipticity as compared with compounds (IV) and (Vb), as is in fact observed (see Table 1).

The brassinosteroid (VI) with an epoxy group in ring A is characterized by a higher value of the molecular ellipticity, which can be explained by considerable conformational changes of ring A. Here, as can be seen from a consideration of molecular models, the C-4 atom passes from the negative left upper sector into the positive lower left sector.

The CD spectra of brassinosteroids (VI), (VIII), and (IXc), each containing an additional chromophore with an optical transition in approximately the same region as that of the carboxy group, deserve separate consideration. In the CD spectrum of compound (VII) with a C=C bond (see Fig. 1) negative and positive maxima are observed at 210 and 235 nm, respectively, with a point of inflection at ~220 nm. In our view, such a complex type of spectrum is possible with the mutual superposition of positive and negative CD bands of approximately the same magnitude having their maxima at ~220 nm. Incomplete compensation of the optical rotation leads to the apparent appearance of two bands of opposite sign close to the rotation maximum. In actual fact, compounds with isolated C=C bonds show CEs of the π_X–π_X* transitions in the 180-220 nm interval [7]. If it is borne in mind that, as in the case of other 7-oxa-6-ketobrassinoids, the carboxylic chromophore will give a positive CE, the C=C bond causes a negative CE.

A complex form of the CD spectrum is also observed in the case of steroid (VIII) with a positive C–Br chromophore in the 3α-position. It is known that alkyl halides show CEs for the n–σ* transitions at the C–Hal bonds, and for the alkyl bromides these are observed at ~207 nm [7]. The mutual superposition of the negative CE for the C–Br chromophore and the small positive CE of the carboxylic chromophore in the spectra of (VIII) give a negative CD band at ~208 nm. It must be mentioned that no such overlapping is observed in the CD spectrum of brassinosteroid (IXc) with the C–Br chromophore in the 3β position. For this compound it is possible to observe only a small lowering of the molecular ellipticity of the n–π* transition of the carboxy group as compared with its analogs (IXa and b) (see Table 1). It is obvious that the optical activity of the n–σ* transition with respect to the C–Br bond strongly depends on its spatial orientation. Brassinosteroid (VIII) contains yet another optically active chromophore – the C=O group – which reveals a strong positive CE (see Table 1). The application of the octant rule to this carbonyl chromophore confirms the positive sign of the CE, since the main part of the molecule is located in the positive upper octant.

EXPERIMENTAL

The CD spectra of the steroids were recorded in the 200-350 nm region on a Jasco J-20 spectropolarimeter in methanolic solution at concentrations of $3 \cdot 10^{-4}$-$1.5 \cdot 10^{-3}$ M in quartz cells with layer thicknesses of 0.2 and 1 cm. The sensitivity of the instrument was 0.005°/cm, the time constant 4, and the rate of scanning 1 nm/min. Molecular ellipticities were determined from three measurements, and the relative error of the ellipticity measurements did not exceed 10%. Absorption spectra were measured on a Specord UV-Vis spectrophotometer at the same concentrations as the CD spectra in cells with layer thicknesses of 1 and 3 cm.

LITERATURE CITED

1. N. V. Kovganko, Khim. Prir. Soedin., No. 2, 159-173 (1991).
2. T. T. Yokota, M. Arima, and N. Takahashi, Tetrahedron Lett., 23, No. 12, 1275-1278 (1982).

3. T. T. Yokota, M. Arima, N. Takahashi, S. Takatsuto, N. Ikekawa, and T. Takematsu, Agr. Biol. Chem., 47, No. 10, 2319-2420 (1983).
4. N. V. Kovganko and Zh. N. Kashkan, Zh. Org. Khim., 26, No. 12, 2545-2552 (1990).
5. S. Takatsuto and N. Ikekawa, Tetrahedron Lett., 24, No. 9, 917-920 (1983).
6. P. Crabbé, An Introduction to the Chiroptical Methods in Chemistry, Offsali, Mexico (1971).
7. W. Klyne and P. M. Scopes, in: Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry, G. Snatzke (ed.), Heyden and Sons, London (1967).

FEATURES OF THE MASS SPECTRA OF HETISINE BASES WITH AN OH GROUP AT C-14

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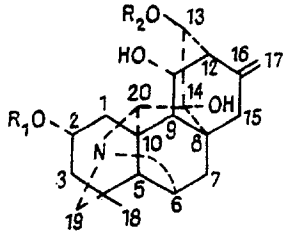
UDC 513.51+547.944/945

The EI mass spectra of five hetisine bases with an OH group at C-14 have been investigated. The main directions of fragmentation are initiated by the cleavage of the C-14–C-20 bond. With the aid of measurements of the elementary compositions of the molecular and fragmentary ions and of a comparison of the B/E linked-scanning and metastable defocusing spectra, the mechanism of the formation of the key fragments has been established and alternative methods for the production of certain ions have been revealed.

We have previously given a detailed discussion of the fragmentation of C_{20}-diterpene bases with the hetisine skeleton [1].

In recent years, a number of publications on the isolation of hetisine alkaloids with substituents in the C13–C14 chain have appeared [2, 3]. In [2], details of the overall mass spectra of 2-acetyl-14-hydroxyhetisine and of 2-isobutyryl-14-hydroxyhetisine and schemes of the fragmentation these bases are given. In the opinion of the authors concerned, the $(M - 28)^+$ ions, the peaks of which are some of the most intense in the spectra, are formed either by the ejection of CH_2N or by the splitting out of a molecule of ethylene from ring B. These processes are accompanied by the elimination of the CO molecule, giving $(M - 56)^+$ ions with the compositions $(M - C_2H_2NO)^+$ and $(M - C_3H_4O)^+$, respectively.

We had available a number of analogous bases with different substituents at C-2 and C-13, and it was therefore of interest to confirm the hypotheses put forward in [2] and also to study the influence of a hydroxy group at C-14 and the nature of the substituents at C-2 and C-13 on the nature of fragmentation under EI and to find alternative methods for the formation of ions with identical masses. For this purpose we used high-resolution mass spectrometry, metastable defocusing (MD) spectra, and B/E = const linked-scanning spectra.



I. $R_1=R_2=H$
II. $R_1=Ac$; $R_2=H$
III. $R_1=COCH_2CH_3$; $R_2=H$
IV. $R_1=COCH(CH_3)_2$; $R_2=H$
V. $R_1=COCH(CH_3)_2$; $R_2=Ac$

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