

MANIFESTATION OF STRUCTURE AND INTERMOLECULAR INTERACTIONS OF BIOLOGICALLY ACTIVE BRASSINOSTEROIDS IN INFRARED SPECTRA

N. A. Borisevich,¹ I. V. Skorniyakov,^{*1}
V. A. Khripach,² G. B. Tolstorozhev,¹
and V. N. Zhabinskii²

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We have analyzed the IR spectra obtained for steroidal phytohormones 24-epibrassinolide, 24-epicastasterone, 28-homobrassinolide, and 28-homocastasterone. The characteristic frequencies of the stretching vibrations of the hydrocarbon groups CH₃, CH₂, and CH and also the C=O groups in the spectra of brassinolides are higher than in the spectra of castasterones, which makes it possible to identify them from the IR spectra. Study of the spectra of these brassinosteroids in different media (pressed samples in KBr, films, solutions in CHCl₃ and CDCl₃) allowed us to establish the presence of intermolecular interactions in which C=O and OH groups, OH–OH groups participate, and also the possible formation of intramolecular hydrogen bonds between the OH groups of the molecules.

Key words: IR spectrum, brassinosteroid, brassinolide, castasterone, characteristic frequencies, intermolecular and intramolecular bonds.

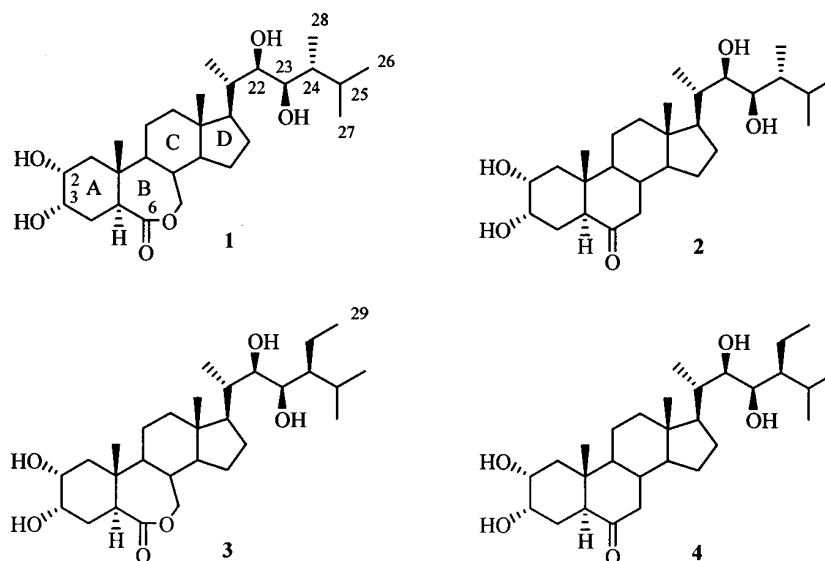
Introduction. Brassinosteroids are a new class of natural and synthetic steroidal phytohormones [1] which play a key role in regulation of the growth and development of plants, and also have good prospects for use in medicine and veterinary practice as new pharmacological agents. A condition for high biological activity of brassinosteroids is the presence in the B ring of a 6-keto (castasterone and related compounds) or 7-oxa-6-keto (brassinolide and other lactones) structural moiety, and also a 2 α ,3 α -diol group on the A ring and a *trans* fusion for the A/B rings (see structural formulas). Along with these structural features, the biological activity of brassinosteroids is determined by the structure of the side chain: the presence and configurations of hydroxyl groups at the carbon atoms C 22 and C 23, and also the nature of the substituent at the carbon atom C 24 [2–4]. It has been established that the greatest biological activity in many test systems is shown by brassinolide, first obtained from pollen of the plant *Brassica napus* [5]. However, a clear interconnection between the structure and the biological properties of brassinosteroids has not yet been established. This makes large-scale use of brassinosteroids in agriculture as plant growth stimulators difficult, and limits their advance into other areas of application.

Despite the fact that the IR spectra provide considerable information about the structure and interactions of molecules, in study of brassinosteroids, IR spectroscopy methods have been used mainly to confirm the course of chemical reactions in synthesis of new compounds in this class.

In this work, we used IR spectroscopy methods to study the structure and intermolecular interactions of 24-epibrassinolide (**1**), 24-epicastasterone (**2**), 28-homobrassinolide (**3**), and 28-homocastasterone (**4**), the structural formulas of which have the form:

^{*}To whom correspondence should be addressed.

¹Institute of Molecular and Atomic Physics (State Scientific Institution), National Academy of Sciences of Belarus, 70 prosp. Nezavisimosti, Minsk 220072. E-mail: lirr@imaph.bas-net.by. ²Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 74, No. 5, pp. 610–616, September–October, 2007. Original article submitted June 11, 2007.



The carbon atoms in the brassinosteroid molecules are located near the plane formed by the rigidly fused rings of the steroid skeleton. The dashed lines in the formulas show that the functional groups or individual atoms, joined by single bonds to the skeleton of the molecule, are located in space below the plane of the molecule. The thick lines mean that the corresponding moieties are located above the plane. So far only brassinosteroids having such a spatial orientation of the OH groups at C 22 and C 23, which is illustrated in the structural formulas (*R* configuration), have been observed in plants, while compounds with the opposite *S* configuration of the OH groups can be obtained only synthetically. Substituents at C 24 of natural brassinosteroids can have both the *R* configuration (compounds **1**, **2**) and the *S* configuration (**3**, **4**).

The Experiment. The studied brassinosteroids were synthesized by the methods described in [6, 7]. They practically do not dissolve in the neutral liquids CCl_4 and CS_2 , which are transparent in the IR region of the spectrum, and so we used CHCl_3 and CDCl_3 . The concentration of brassinosteroids was no greater than 0.01 M in these solvents. The cuvet thickness was 0.43 mm. Films of the studied substances were obtained by evaporation of solutions of the compounds in CHCl_3 on NaCl windows at room temperature and normal atmospheric pressure. The synthesized brassinosteroid substances were ground with anhydrous, finely dispersed KBr, and the prepared powder was pressed under vacuum according to the standard procedure. The concentration of the studied substances, pressed in KBr, was no greater than 0.1 mass %.

The IR spectra of the brassinosteroid samples were recorded on a NEXUS IR Fourier spectrometer with OMNIC software in the range $4000\text{--}400\text{ cm}^{-1}$ with spectral resolution 2 cm^{-1} and 320 repeated scans. For accurate determination of the frequencies of the maxima (ν_{max}) of the absorption bands in the IR spectra of the brassinosteroids, the contours of broadened and asymmetric bands were also separated into components by differentiation of the spectral curves, with analysis of the second derivatives ($d^2T/d\nu^2$).

Results and Discussion. The studied brassinosteroids have similar chemical structures: two hydroxyl groups on the C 2 and C 3 carbon atoms of ring A, and two hydroxyl groups with the *R* configuration on the side chain for hydrocarbon atoms C 22 and C 23, identical structure of rings A, C, D. However, there are also substantial differences in the structure of the B ring, the substituents on the C 24 atom, and their configurations.

Figure 1 shows the IR spectra in a broad range of frequencies for four compounds from the brassinosteroid class. The frequencies of the maxima for the absorption bands of the characteristic groups of atoms are shown in Table 1. In the high-frequency region of the IR spectra of brassinosteroids ($3490\text{--}3420\text{ cm}^{-1}$), there are broad bands involving participation of stretching vibrations of associated OH groups. Brassinosteroids are in the crystalline state in KBr.

In the structure of brassinosteroids, the hydrocarbon groups CH_3 , CH_2 , and CH are found on different rings and on the side chain. In this case, epibrassinosteroids differ from homobrassinosteroids in that a CH_3 group with an *R* configuration is bonded to the C 24 atom of epibrassinosteroids, while a $\text{CH}_2\text{--CH}_3$ group with an *S* configuration is involved for homobrassinosteroids; brassinolides differ from castasterones in the different bonding of the CH_2 group

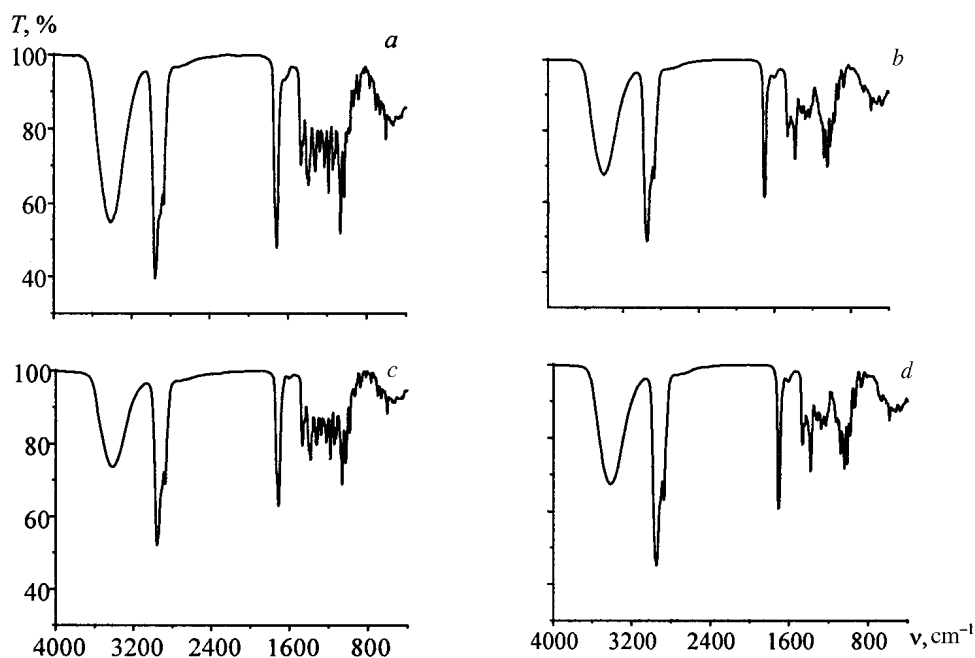


Fig. 1 IR spectra of 24-epibrassinolide (a), 24-epicastasterone (b), 28-homobrassinolide (c), and 28-homocastasterone (d), pressed in KBr.

to adjacent atoms of the *B* ring. The CH_2 and CH groups in the same molecule are not equivalent. In the IR spectra of the studied brassinosteroids pressed in KBr, the absorption in the $3000\text{--}2800\text{ cm}^{-1}$, consisting of a series of bands (Fig. 1), belong to stretching vibrations of the hydrocarbon groups. In Fig. 2, this region is shown on a larger scale, and the contours of the spectral absorption bands are differentiated: $d^2T/d\nu^2$ (curves 2).

In the absorption spectra of the studied brassinosteroids, there are five bands each belonging to stretching vibrations of the hydrocarbon groups. The most high-frequency strong (s) bands is assigned to antisymmetric vibrations of the CH_3 groups. One significantly overlaps with another band (also strong), which is assigned to antisymmetric vibrations of the CH_2 groups. The third band of medium intensity (m) belongs to the stretching vibrations of the CH groups. The last two bands (the fourth band (m) and the fifth weak band (w)) are assigned to symmetric stretching vibrations of the CH_3 and CH_2 groups respectively. The overlapping bands of the antisymmetric stretching vibrations of these groups in brassinosteroids form a band with a characteristic contour. In the case of brassinolides, the intense band of the CH_3 groups and the frequency of the maximum for the overall band is equal to 2960 cm^{-1} while in the corresponding spectra of castasterones, the intense band for the CH_2 groups and the common maximum has a lower frequency: 2947 cm^{-1} .

Significantly more information can be extracted from analysis of the differential spectra, shown in Fig. 2 (curves 2). The frequencies of the maxima for the bands and their assignment to the stretching vibrations of the CH_3 , CH_2 , and CH groups are shown in Table 1.

In the structure of the studied brassinosteroids, there is an equal number of CH_3 groups and they are joined to the skeleton of the molecules by identical $\text{C}\text{--}\text{CH}_3$ bonds. However, besides the bands belonging to antisymmetric and symmetric vibrations of these groups with practically indistinguishable frequencies $\nu_{\text{as}} \approx 2970\text{--}2967\text{ cm}^{-1}$ and $\nu_{\text{s}} \approx 2870\text{--}2868\text{ cm}^{-1}$, in the differential spectra of brassinolides there is one more band $\nu_{\text{as}} = 2997\text{ cm}^{-1}$.

In brassinosteroid molecules, there are seven CH_2 groups each included in the ring, while for homobrassinosteroids there is one more group each on the side chain. If all the CH_2 groups were functionally identical, then in the IR spectra of each compound we would observe one band each for their antisymmetric and symmetric stretching vibrations. In the differential spectra (Fig. 2), two bands each, ν_{as} and ν_{s} , belong to these groups (Table 1). The $\text{O}=\text{C}\text{--}\text{O}$ group has a substantial effect on vibrations of the CH_2 group that are part of the seven-membered *B* ring of brassinolides, while the $\text{C}=\text{O}$ group has a substantial effect on the CH_2 group that is part of the six-membered *B* ring of brass-

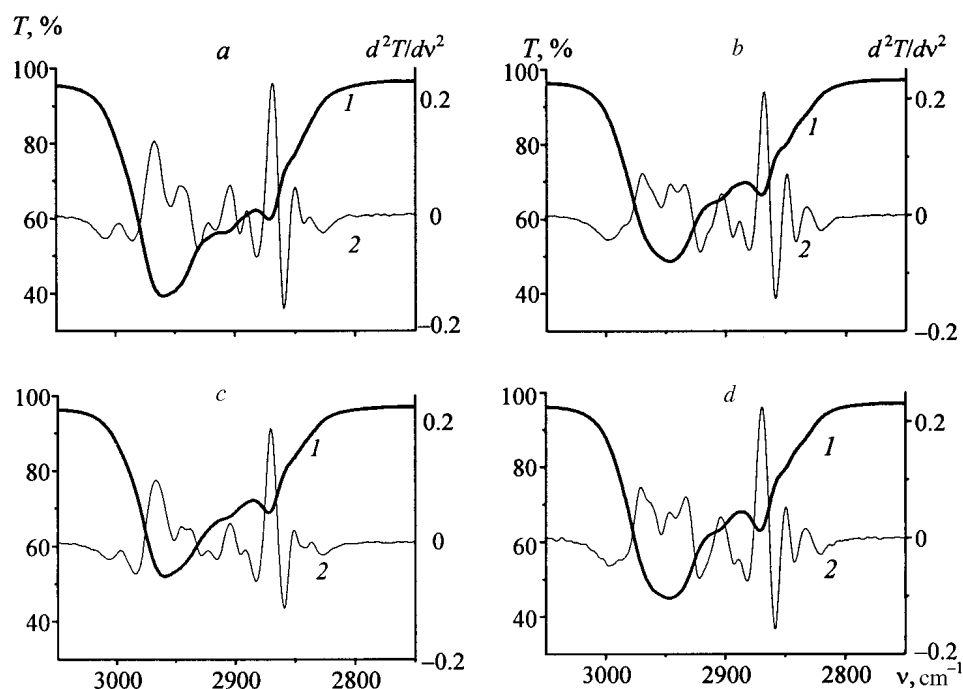


Fig. 2 IR spectra (1) and their second derivatives (2) in the region of the stretching vibrations of the CH₃, CH₂, and CH groups of 24-epibrassinolide (a), 24-epicastasterone (b), 28-homobrassinolide (c), and 28-homocastasterone (d), pressed in KBr.

TABLE 1 Frequencies of Maxima (ν_{\max} , cm⁻¹) for IR Absorption Bands for Stretching Vibrations of Groups of Atoms in Brassinosteroids Pressed in KBr

Characteristic group	Type of vibration	24-Epibrassinolide	24-Epicastasterone	28-Homobrassinolide	28-Homocastasterone
OH	$\nu(\text{OH})$	3413	3405	3415	3408
CH ₃	$\nu_{\text{as}}(\text{CH})$	2997	—	2997	—
	$\nu_{\text{s}}(\text{CH})$	2967	2970	2967	2970
CH ₂	$\nu_{\text{as}}(\text{CH})$	2868	2868	2870	2869
		2945	2946	2945	2946
	$\nu_{\text{s}}(\text{CH})$	2938	2934	2938	2933
		2850	2849	2851	2849
CH	$\nu(\text{CH})$	2838	2833	2836	2833
		2922	—	2923	—
		2904	2903	2904	2903
C=H	$\nu(\text{C}=\text{O})$	2890	2889	2891	2889
		1735	1714	1736	1714
C—O—C=O	$\nu(\text{C}=\text{O})$	1710	1698	1712	1698
		1184	—	1185	—

inolide. This is confirmed by the higher values of the frequencies $\nu_{\text{as}} = 2938 \text{ cm}^{-1}$ for brassinolides than for the corresponding frequencies $\nu_{\text{as}} \approx 2933 \text{ cm}^{-1}$ for castasterones. We know that a direct bond between the functional groups and the oxygen atom increases their vibrational frequencies. The relationship between the symmetric vibrations of these groups is similar in the spectra of brassinolides and castasterones. The bands for the stretching vibrations of the re-

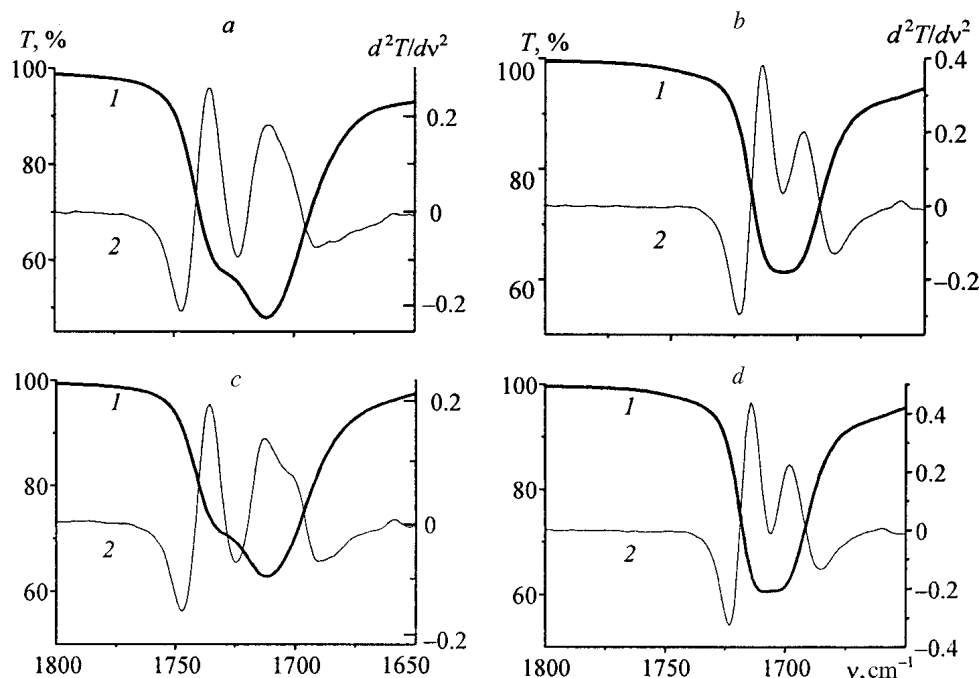


Fig. 3 IR spectra (1) and their second derivatives (2) in the region of the stretching vibrations of the C=O groups of 24-epibrassinolide (a), 24-epicastasterone (b), 28-homobrassinolide (c), and 28-homocastasterone (d), pressed in KBr according to the standard procedure.

TABLE 2 Frequencies of Maxima (ν_{\max} , cm^{-1}) for IR Absorption Bands for Stretching Vibrations of C=O Groups in IR Spectra of Brassinosteroid Solutions and the Solid Phase

Compound	Solution			Film	Pressed in KBr
	in CHCl_3		in CDCl_3		
	0.01 M	0.001 M	0.004–0.001 M		
24-Epibrassinolide	1721	1720	1719	1729	1735
	–	–	–	1709	1710
24-Epicasterone	1704	1704	1704	1709	1714
	–	–	–	1698	1698
28-Homobrassinolide	1720	1721	1720	1731	1736
	–	–	–	1711	1712
28-Homocasterone	1703	1704	1704	1713	1714
	–	–	–	1700	1698

maining CH_2 groups that are part of the structure of the studied molecules, also including the CH_2 of the side chain of homobrassinosteroids, have practically identical frequencies for both ν_{as} and ν_{s} (Table 1).

The CH groups in the structure of brassinosteroids are also not spectrally equivalent. Four of them are bonded to OH groups of the A ring and the side chain, five are found in the rings, three are bonded to CH_3 groups on the side chain, and for homobrassinosteroids, one is joined to a CH_2 group. In the differential spectra of brassinolides, we observe three bands, while for castasterones we observe two bands, assigned to the stretching vibrations of these groups. We can hypothesize that the bands with frequency of the maximum $\nu = 2904 \pm 1 \text{ cm}^{-1}$ are assigned to stretching vibrations of CH groups in H-C-OH , and the lower-frequency bands, also having practically identical frequencies for all the studied brassinosteroids, belong to the rest of the CH groups. Then the high-frequency bands $\nu =$

TABLE 3 Frequencies of Maxima (ν_{\max} , cm^{-1}) for Absorption Bands for Stretching Vibrations of OH Groups in IR Spectra of Brassinosteroid Solutions and the Solid Phase

Compound	Solution		Film	Pressed in KBr
	in CHCl_3	in CDCl_3		
	0.002 M	0.002 M		
24-Epibrassinolide	3623	3621	–	–
	3581	3586	–	–
	–	–	3421	3413
24-Epicasterone	3624	3624	–	–
	3574	3577	–	–
	–	–	3414	3405
28-Homobrassinolide	3621	3623	–	–
	3584	3581	–	–
	–	–	3430	3415
28-Homocasterone	3621	3620	–	–
	3582	3583	–	–
	–	–	3425	3408

$2922 \pm 1 \text{ cm}^{-1}$ in the spectra of epibrassinolides and homobrassinolides should be assigned to CH groups bonded to the seven-membered *B* ring.

From analysis of the data obtained, it follows that the IR spectra of brassinolides in the region of the stretching vibrations of the CH_3 , CH_2 , and CH groups are significantly different from the spectra of castasterones. These differences in the spectra should be associated with the influence of the structure and configuration of the *B* ring on the hydrocarbon groups added to it.

Within the structure of the molecules of the studied brassinosteroids, there is one carbonyl group each on the *B* ring. Intense, complex bands in the IR spectra of brassinosteroids pressed in KBr, $\nu_{\max} \approx 1710 \text{ cm}^{-1}$ (Fig. 1), belong to stretching vibrations of these groups. In the absorption spectra on a larger scale and the differential spectra (curves 2), shown in Fig. 3, we observe two bands each, belonging to the stretching vibrations of the C=O groups. They also occur in the spectra of films of these compounds. The frequencies of their maxima are given in Table 2. Compounds **1**, **2** and especially **3**, **4** dissolve poorly even in the polar solvents CHCl_3 and CDCl_3 . The maximum concentration of the solutions was 0.01 M. At these and lower concentrations, in the spectra of solutions of all the studied brassinosteroids we observe one band each for the stretching vibrations of the C=O groups (Table 2).

From analysis of the data obtained, we can draw the following conclusions. The low-frequency bands in the IR spectra of the solid phase belong to the stretching vibrations of associated C=O groups. They can engage in a hydrogen bond with the OH groups. The absence of these bands in the spectra of the solutions is proof for an intermolecular hydrogen bond $\text{C=O} \cdots \text{H-O}$ but not for an intramolecular hydrogen bond. In all the studied media, the frequencies of the vibrations of the C=O groups of the brassinolides are higher than the corresponding frequencies of the castasterones, which is associated with the influence of the oxygen of the seven-membered *B* ring.

Usually the frequencies of the stretching vibrations of free C=O groups of the compounds are higher in solutions than in the solid phase. In this case, the relationship between the corresponding frequencies is the reverse (Table 2). This is connected with formation of weak associates between the oxygen of the carbonyl group and the hydrogen or deuterium of the polar solvents: $\text{C=O} \cdots \text{HCCl}_3$ and $\text{C=O} \cdots \text{DCCl}_3$.

In the IR spectra of the films and the brassinosteroids pressed in KBr, we observed one broad absorption band each, $\nu_{\max} \approx 3430\text{--}3405 \text{ cm}^{-1}$, assigned to associated OH groups. The frequencies of their maxima ν_{\max} are given in Table 3. They are $8\text{--}17 \text{ cm}^{-1}$ higher in the spectra of the films, due to the different physical properties of the films and the pressed samples. For both media, the values of ν_{\max} for these bands are higher in the spectra of the brassinolides. In the spectra of solutions of brassinosteroids in CHCl_3 and CDCl_3 at concentrations of 0.002 M or lower, we recorded two narrow bands each, with significantly higher frequencies ν_{\max} than in the spectra of the solid

phase. The highest frequency band has the same value $\nu_{\max}^1 = 3622 \pm 1 \text{ cm}^{-1}$ in the spectra of all the studied brassinosteroids. The frequency of the maxima for the second band is lower than for the first by $\approx 40 \text{ cm}^{-1}$ and all the compounds have close values for this frequency: $\nu_{\max}^2 = 3581 \pm 3 \text{ cm}^{-1}$ (see Table 3). The spectra of solutions of brassinosteroids in CHCl_3 and CDCl_3 are practically identical. In these solutions, associates of H and D with the OH groups of the brassinosteroids can form, but they are weaker than their associates with the C=O groups.

The brassinosteroid structure has two OH groups each on the A ring, located below the plane of the molecule, and two on the side chain. Broad bands with $\nu_{\max} \approx 3430\text{--}3405 \text{ cm}^{-1}$ in the spectra of the solid phase of the brassinosteroids are assigned to OH groups forming an intermolecular hydrogen bond, since they are missing in the spectra of the solutions. From x-ray diffraction analysis of the crystals of the epicastasterone derivative, whose structure has only two OH groups, intermolecular hydrogen bonds are formed in them between atoms of the group on the A ring of one molecule and the OH group of the side chain of another molecule. The carbonyl group does not participate in the association [8]. In the studied brassinosteroids, intermolecular hydrogen bonds are formed both between the OH groups and with the C=O groups, since in the spectra of the solid phase of the brassinosteroids we observe bands not only for free carbonyl groups but also for ones bound to an OH group. The overtones of the carbonyl groups also participate in formation of the broad bands $\nu_{\max} \approx 3430\text{--}3405 \text{ cm}^{-1}$. Using an IR microscope, we obtained the IR spectra of brassinosteroid crystallites. In each of these broad, structureless bands, we see 2–3 poorly resolved bands.

The high-frequency bands ν_{\max}^1 and ν_{\max}^2 in the spectra of brassinosteroid solutions are assigned to OH groups not participating in intermolecular hydrogen bonds. The hydroxyl groups on the A ring are sufficiently close and can form an intramolecular hydrogen bond, as a result of which a band appears in the spectrum with frequency ν_{\max}^2 , $\approx 40 \text{ cm}^{-1}$ lower than the frequency of the free OH groups $\nu_{\max}^1 = 3622 \pm 1 \text{ cm}^{-1}$. However, the OH groups that are part of the A ring and the side chain are not equivalent. We can hypothesize that the band with frequency ν_{\max}^1 is assigned to free OH groups of the side chain, while ν_{\max}^2 is assigned to free OH groups of the A ring. In order to unambiguously establish the nature of these bands, we need to carry out studies of the IR spectra of brassinosteroids with different numbers and positions of the hydroxyl groups within their structures.

Conclusion. We have established a number of spectral signs allowing us to identify 24-epibrassinolides, 28-homobrassinolides, and 24-epicastasterones, 28-homocastasterones. The structures of these brassinosteroids contain an identical number of CH_3 and CH groups, practically identically bonded to the skeleton of the molecule. In the IR spectra of the brassinolides, besides the bands with identical frequencies for these groups, for all the studied compounds there are bands with higher frequencies, $\nu_{\text{as}} = 2997 \text{ cm}^{-1}$ (CH_3) and $\nu = 2922 \text{ cm}^{-1}$ (CH). It is only in the spectra of the brassinolides that we observe bands with $\nu = 1184 \text{ cm}^{-1}$ for stretching vibrations of the C–O group that is part of the C–O–C=O group on the seven-membered B ring. The frequencies of the stretching vibrations for the C=O groups are also higher for the brassinolides. These spectral differences are associated with the structural and configurational features of the B rings of brassinolides and castasterones.

In the IR spectra of brassinosteroids pressed in KBr and their films, we recorded two bands each, assigned to stretching vibrations of the carbonyl groups: the high-frequency bands belong to free C=O groups, while the low-frequency bands belong to the hydrogen bond formed with the OH groups. In these media, we observe only bands for bound OH groups.

In the spectra of solutions of brassinosteroids in CHCl_3 and CDCl_3 , there is one band each for the free C=O groups, while two bands each belong to the OH groups, the corresponding frequencies of which $\nu_{\max}^1 = 3622 \pm 1 \text{ cm}^{-1}$ and $\nu_{\max}^2 = 3581 \pm 3 \text{ cm}^{-1}$ are identical for the brassinolides and castasterones. The high-frequency band ν_{\max}^1 is assigned to stretching vibrations of free OH groups on the side chain, while ν_{\max}^2 should be assigned to the OH groups on the A ring, forming an intramolecular hydrogen bond.

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