

CIRCULAR DICHROISM SPECTRA OF STIGMASTANE 3 β -BENZOYLOXY-6-KETOSTEROIDS

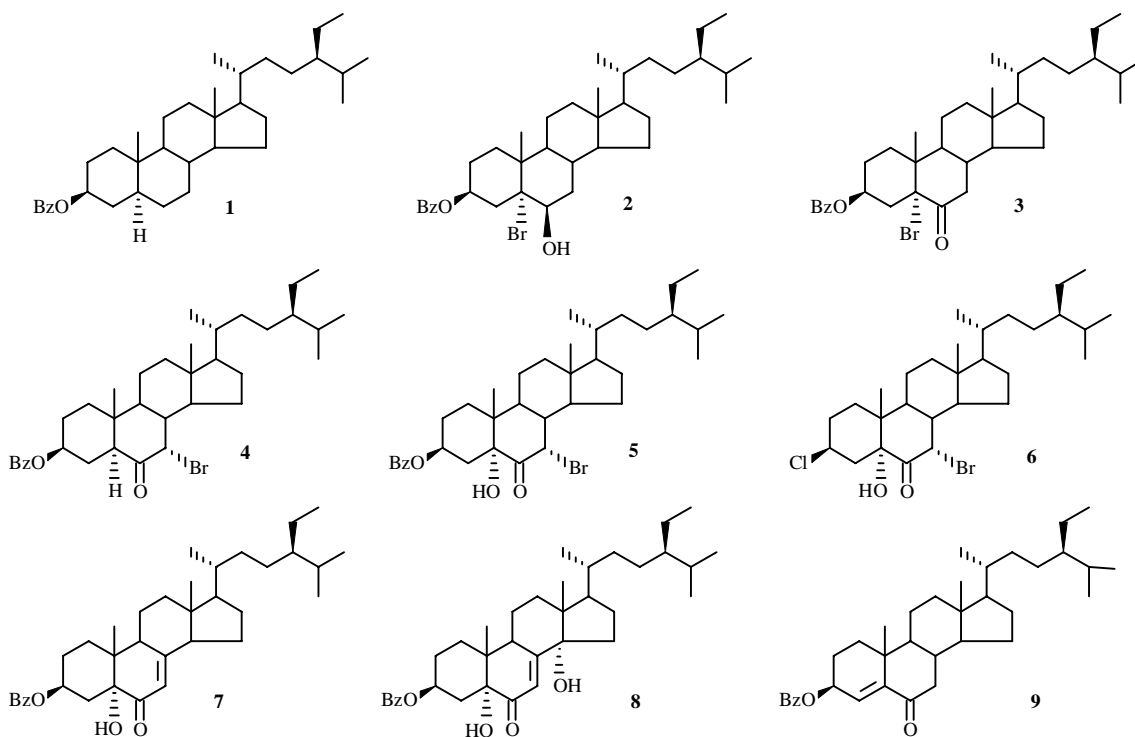
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CD spectra of stigmastane derivatives containing a 3 β -benzoyloxy group and a saturated or conjugated 6-ketone were studied. Application of known empirical rules to functional groups enabled the mutual influence on the optical activity of their electronic transitions in the compounds to be evaluated. It has been demonstrated that CD spectra can be used to prove the structures of these steroids.

Key words: ecdysteroids, 3 β -benzoyloxy-6-ketosteroids, circular dichroism.

Ecdysteroids are a numerous group of natural compounds that possess a unique set of structural elements that distinguish them from other natural polyhydroxysteroids [1-3]. About 300 ecdysteroids have been isolated from invertebrates, plants, and fungi. Modern spectral methods must be used as a rule to establish their structures owing to the exceedingly complicated ecdysteroid molecules. One of these methods is circular dichroism (CD) spectroscopy, which is widely used in the chemistry of ecdysteroids. CD spectra can reliably prove the presence in these molecules of a Δ^7 -6-ketone and *cis*-fusion of rings A and B. Furthermore, CD spectra are used to determine the stereochemistry of the OH groups in ecdysteroids by converting them to benzoates [4]. It should be noted that ecdysteroid benzoates have also been identified as natural compounds [5-14].



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TABLE 1. Band Parameters in UV Spectra and CD Spectra of Steroids **1-9**

| Compound | UV spectrum | | CD spectrum | |
|----------|---------------------|-----------------------|---------------------|---|
| | λ , nm | ϵ , L/mol·cm | λ , nm | Sign and $[\theta] 10^4$, deg·cm ² /mol |
| 1 | 230 | 9.800 | 230 | -6.5 |
| | 273 | 500 | | |
| 2 | 230 | 14.500 | 227 | 3.5 |
| | 273 | 800 | | |
| 3 | 215 | 15.000 | 220 | 17.5 |
| | 275 | 900 | | |
| | 310 | 80 | 308 | -21.0 |
| 4 | 230 | 13.400 | 225 | 6.5 |
| | 265 | 800 | | |
| | 308 | 80 | 308 | 11.0 |
| 5 | 229 | 15.500 | 225 | 7.0 |
| | 273 | 800 | | |
| | 307 | 70 | 300 | -7.0 |
| 6 | 200-270 (band tail) | - | 200-250 (band tail) | - |
| | 335 | 90 | 332 | 7.5 |
| 7 | 232 | 4.700 | 220 | 11.0 |
| | 245 (shoulder) | 2.200 | 245 | -22.0 |
| | 330 | 50 | 337 | 6.5 |
| 8 | 233 | 3.600 | 225 | 9.5 |
| | 255 (shoulder) | 1.500 | 245 | -18.0 |
| | 340 | 70 | 343 | 7.5 |
| 9 | 231 | 18.000 | 210 | 18.0 |
| | | | 238 | -20.0 |
| | 320 | 30 | 322 | -3.5 |

We previously synthesized [15, 16] several structural analogs of ecdysteroids with structures similar to physanols A and B, which were isolated from *Physalis franchetti* [14]. These molecules typically have 3 β -benzoyloxy and Δ^7 -6-ketone groups. Some of the compounds prepared by us and intermediates in their synthesis exhibited significant insecticidal activity for Colorado-beetle larvae [17].

Herein we present results from a CD study of steroids **1-9**, which we previously synthesized, in order to establish the possibility in principle of the method to identify the structures of such compounds, which contain simultaneously two closely situated chromophores, the 3 β -benzoyloxy and 6-ketone groups. The 6-ketone can be saturated or unsaturated or occupy the α -position relative to a OH or Br. Table 1 lists the CD spectral parameters of benzoyloxystigmastanes **1-5** and **7-9** and chlorostigmastanone **6** and UV spectral data. Figure 1 shows for the sake of illustration CD spectra of certain characteristic steroid benzoates.

Most steroid benzoates contain a saturated or conjugated 6-ketone, the absorption and optical rotation of which may overlap with analogous spectral characteristics of the benzoyloxy group. Of the two bands in absorption spectra of **1** and **2**, which contain the benzoate chromophore, only the stronger band near 230 nm possesses optical activity. This is due to the $\pi-\pi^*$ transition of the benzoyloxy group (Table 1). The 3 β -benzoyloxy and 6-ketone are located close to each other and can, like other proximal substituents, mutually affect the optical activity of electronic transitions of each of these chromophores. Therefore, the contribution of the separate structural fragments to the optical rotation can be estimated by applying the corresponding empirical rules to the functional groups.

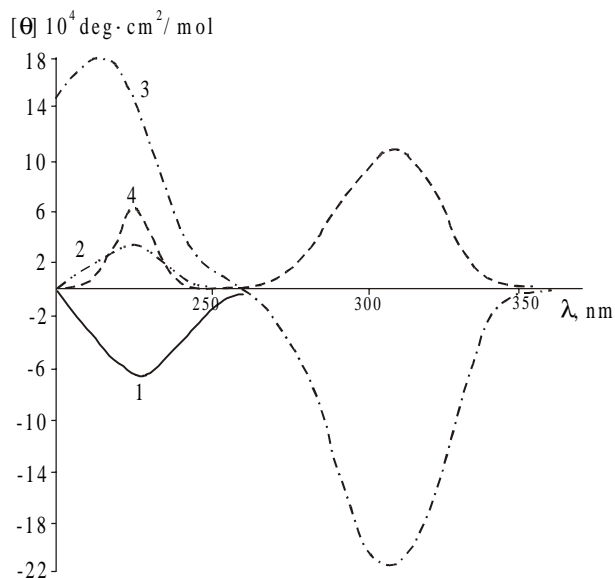


Fig. 1. CD spectra of steroid benzoates **1-4**. Curve numbers correspond to compound numbers.

For benzoates, the sector rule is applicable [4, 18], according to which the space occupied by the molecule is divided into four sectors by symmetry planes A and B and then into eight sectors by additional planes C and D, which are perpendicular to plane A and pass through both oxygen atoms (Fig. 2). The preferred orientation of the benzoyloxy group is the conformation fixed between the H atom on the C atom with the ester and the smallest substituent. For our compounds with the preferred benzoyloxy conformation, the carbonyl oxygen is limited by the axial H on C-3 and the equatorial H on C-2. If the benzoate chromophore is examined from the *para*-position, the contributions to the Cotton effect (CE) of its $\pi-\pi^*$ transition near 230 nm from adjacent α,β - and β,γ -bonds can be estimated as a function of their placement in a sector of the corresponding sign (Fig. 2). The contribution of double bonds are considered to be more substantial because of their higher polarizability than simple C-C bonds.

The direct octant rule [18], which relates the sign and magnitude of the CE of the $n-\pi^*$ transition in the chromophore to its asymmetric environment, is applicable to a saturated ketone. The sign of the $n-\pi^*$ transition of conjugated transoid enones obeys the inverse octant rule [18], according to which the space occupied by the molecule is divided by three mutually perpendicular planes passing through the C=O bond. These are the symmetry planes of orbitals involved in the $n-\pi^*$ transition that divide the bond by halves into four front and four rear octants (if the chromophore is examined from the side of the oxygen atom). The contribution to the optical rotation of the chromophore of substituents adjacent to it can be estimated from their location in octants of the corresponding signs (Fig. 3).

In our opinion, it is more correct in applying the octant rule to benzoates to consider the location in the sectors of the corresponding signs not of the chemical bonds next to the chromophore but, like for other empirical rules, of neighboring C atoms and heteroatoms, especially the location of asymmetric centers in the molecule.

Application of the sector rule to the steroidal derivative **1**, which has the simplest structure and contains only the benzoyloxy group, showed that the negative sign of the CE for the $\pi-\pi^*$ transition of the chromophore at 230 nm is due to predominance of negative contributions of asymmetric C atoms C-3 and C-10 with angular methyl C-19, located in the negative lower right and negative upper right sectors, respectively, over the positive contributions of C-1 and C-2, which lie in the positive upper right sector, and C-4, C-5, and C-6, occupying the positive lower right sector. The rotation of the CE sign in the spectrum of 3β -benzoyloxy- 5α -bromo- 6β -hydroxystigmastane **2** (Fig. 1) is due to the significant positive contribution of the axial Br [19] and, obviously, to a lesser extent the 6β -hydroxyl, located in the positive lower right sector.

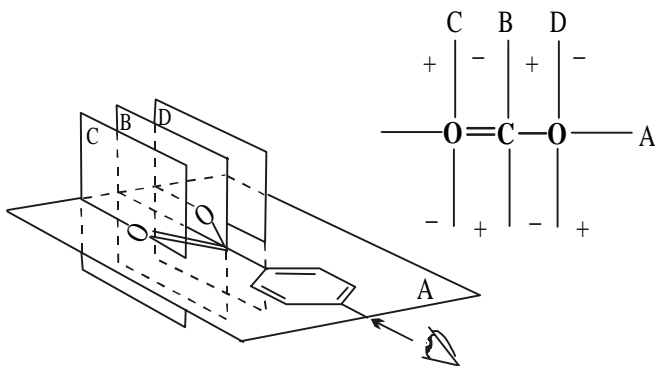


Fig. 2

Fig. 2. Sector rule for benzoates and sector signs.

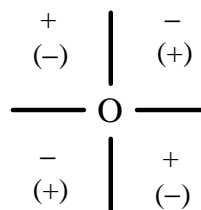


Fig. 3

Fig. 3. Signs of rear octants in direct and inverse (in parentheses) octant rules for the carbonyl.

Steroidal benzoates **3**, **4**, and **5**, which contain saturated 6-ketones, exhibit in the long-wavelength region of the spectrum strong CE for the $n-\pi^*$ transition of the ketone chromophore (Table 1). Its sign in the spectra of the first two compounds, like in spectra of α -bromo-substituted 6-ketosteroids examined by us previously [20], depends on the position of the axial Br next to the ketone. Use of the octant rule for the ketone found that the negative sign of the CE at 308 nm in the spectrum of **3** is due to the location of the Br atom in the lower left rear octant (Fig. 3) whereas the position of the 7α -Br in **4** in the lower right rear octant leads to a reversal of the CE sign.

3β -Benzoyloxyketosteroid **5** has a negative CE at 300 nm. The spectrum of its 3β -chloro analog (**6**) exhibits a positive long-wavelength CE of about the same strength (Table 1). The differences in the CE sign are undoubtedly related to the contribution of the 3β -substituent to the optical rotation of the 6-ketone. Molecular models of the compounds and the octant rule show that the 3β -chloro substituent lies in the positive lower left front octant whereas the 3β -benzoyloxy group, except for the oxygen atom bound to the steroid ring, is situated in the negative upper left front octant. The negative contribution of the 3β -benzoyloxy group to the CE of the $n-\pi^*$ transition of the saturated 6-ketone is evident also in comparing the molecular ellipticity of the long-wavelength band in 3β -benzoyloxy-5-bromo- and 3β -benzoyloxy-7-bromo-6-ketosteroids **3** and **4** (Table 1) and in comparing the $[\theta]$ values for these compounds and their 3β -chloro analogs studied by us previously, with $[\theta]$ of $-17.0 \cdot 10^4$ and $12.5 \cdot 10^4$ deg \cdot cm 2 /mol, respectively [20].

Actually, the molecular ellipticity of the negative long-wavelength band in **3** is greater and $[\theta]$ of the positive long-wavelength band in **4** is lesser than the $[\theta]$ values given here for the band in the corresponding 3β -chloro analogs. The fact that the molecular ellipticity of the negative long-wavelength band in **3** is almost two times greater than its value for the positive band in **4** is also logically explained by the negative contribution of the 3β -benzoyloxy group rather than such a large difference of contributions from the axial Br on C-5 and C-7.

The contribution of the saturated 6-ketone to the CE of the $\pi-\pi^*$ transition of the 3β -benzoyloxy group can be estimated by comparing its strength in spectra of **3-5** and **2**, which has no 6-ketone. The significant increase in the strength of the positive CE of the $\pi-\pi^*$ transition and its short-wavelength shift to 215 nm in the spectrum of **3** (Fig. 1, curve 3) are due not only to the superposition of the positive tail of the band due to $\pi-\pi^*$ and $n-\sigma^*$ transitions of the ketone chromophore (with a sign opposite to that of the band for the $n-\pi^*$ transition [8, 21]) but also to the positive contribution of the ketone, which is stronger than that of hydroxyl, which lies according to the sector rule in the positive lower right sector. The positive contribution of the 6-ketone to the CE of the $\pi-\pi^*$ transition of the 3β -benzoyloxy group is seen more clearly in the spectrum of **4**. The molecular ellipticity of the band at 230 nm increases relative to that in the spectrum of **2** (Fig. 1, curves 4 and 2) on the background of the negative tail of the $\pi-\pi^*$ and $n-\sigma^*$ transitions of the ketone chromophore and of the positive contribution of the more distant axial Br on C-7, which is obviously less significant than in **3**. The rather high molecular ellipticity of the band at 229 nm in the spectrum of **5** (Table 1) with an expected (on the basis of the relatively small value of $[\theta]$ for the band at 300 nm) weak positive tail for the $\pi-\pi^*$ and $n-\sigma^*$ transitions of the ketone chromophore can also affect the positive contribution of the 6-ketone to the CE of the benzoate chromophore.

The lack of the required set of steroidal benzoates with a conjugated ketone prevented us from unambiguously estimating the mutual influence of the chromophores on their optical activity. The counterclockwise position of the C=O and double bond in steroidal benzoates **7** and **8**, which follows from an analysis of molecular models of the compounds, leads to the conclusion that the transoid Δ^7 -en-6-ketone chromophore in these compounds obeys the enone chirality rule, the inverse octant rule is applicable to its $n-\pi^*$ transition [18] and confirms the higher $[\theta]$ value of the long-wavelength band in **8** than in **7** (Table 1). This is due to the positive contribution of the axial hydroxyl on C-14, which lies in the upper right rear octant. According to the inverse octant rule, a 3β -benzoyloxy group lying in the upper left front octant should have a positive contribution to the CE of the band for the $n-\pi^*$ transition of a conjugated transoid 6-ketone chromophore (Fig. 3). However, this contribution cannot be estimated experimentally because the positive sign of the CE for the $n-\pi^*$ transition of the ketone chromophore should be defined mostly by the adjacent axial hydroxyl, which lies in the positive lower left rear octant. According to Snatzke [21], the direct octant rule is applicable to the $n-\pi^*$ transition of the cisoid $\pi\Delta^4$ -en-6-ketone chromophore in **9**. Therefore, the negative sign of its CE is consistent with a possible contribution of the 3β -benzoyloxy group.

According to the sector rule for benzoates, a conjugated 6-ketone chromophore, analogously to a saturated ketone chromophore, should have a positive contribution to the CE of the $\pi-\pi^*$ transition of a 3β -benzoyloxy group. However, it is difficult to estimate its contribution because of the presence of two strong bands of opposite signs (Table 1) in the CD spectra of steroidal benzoates **7-9** in the range 210-240 nm. These are due to the $\pi-\pi^*$ transition of conjugated ketone chromophores.

Thus, the CD spectroscopic investigation proves the presence in 3β -benzoyloxy-6-ketosteroids of the introduced functional groups and enables additional information about the structures to be obtained. The information is usually obtained using traditional spectral (NMR, IR, mass) methods. Consequently, it is hoped that CD spectra will be used more extensively to assign structures in this series of steroids.

EXPERIMENTAL

The studied compounds were synthesized as before [15, 16]. CD spectra were recorded on a J-20 spectropolarimeter (Japan) in ethanol at concentrations of $0.9-8.0 \cdot 10^{-4}$ M in quartz cuvettes 0.2- and 1.0-cm thick. The sensitivity of the instrument was 0.005 deg/cm; time constant, 4; scan rate dependent on spectral region, 5 and 10 nm/min. The molecular ellipticity was determined with a relative uncertainty of <20%. UV absorption spectra were measured on a Specord M-400 spectrophotometer (Germany) under standard conditions in quartz cuvettes 0.2- and 1.0-cm thick at the same solution concentrations as for the CD spectra. The relative uncertainty in the molar extinction coefficients was <10%.

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