Chemistry of Natural Compounds, Vol. 30, No. 3, 1994

CIRCULAR DICHROISM AND STEREOCHEMISTRY OF THE C-20 AND C-22 ASYMMETRIC CENTERS OF 17-ISOXAZOLINYL- AND 20-HYDROXY-20-ISOXAZOLINYLSTEROIDS*

N. I. Garbuz, G. S. Yankovskaya, A. V. Baranovskii, R. P. Litvinovskaya, and V. A. Khripach

UDC 547.92:547.963

The CD spectra of a number stereoisomeric isoxazolinylsteroids have been investigated. Starting from established rules, it has been shown that this method can be used to determine the configurations of the C-20 and C-22 centers of the 17- and 20-isoxazolinylsteroids studied.

The epimeric 20-hydroxy-20-isoxazolinylsteroids (**1-7**) have been studied previously [1, 2], their configurations at C-22 being established on the basis of differences in their PMR spectra and the assumption of a predominant conformation for each isomer. The stereochemisry of one (the main one) of the epimers of (**1**) was established by x-ray structural analysis, and it was identified as the 22R- epimer [3]. The determination of the configurations of the individual centers of isoxazolinyl-steroids by simple and accessible methods is a very urgent task. We have already shown that these compounds are convenient intermediates in the synthesis of the complexly functionalized side-chains of ecdysteroids [4], brassinosteroids [5] and their analogs [6], and the sapogenins of a number of starfish [7] and soft corals [8].

In the present paper we consider the CD spectra of a number of stereomeric 20-isoxazolinylsteroids (**1-11**) and also of 17-isoxazolinylsteroids related to them (**12-16**), the structures of which we have described previously [9], with the aim of finding regular characteristics in their CD spectra according to the configurations of the C-22 and C-20 centers, respectively, and to structural features of the compounds. The results of CD measurements, together with details of UV spectra, are given in Table 1. The CD spectra of some compounds typical of those that we have considered are shown in Fig. 1. We may note, above all, that all the stereomeric pairs that we have considered (**1-2**, **3-4**, **5-6**, **9-10**, **12-13**, and **15-16**) are characterized by opposite signs of their Cotton effects (CE) in the 212-220 nm region, which makes it possible to identify spatial isomers from their CD spectra.

For stereochemical assignments in steroids with isoxazoline rings it is possible to use an empiricial rule connecting the sign of the band of the n-π^* transition of the C=N bond in cyclic azomethines with the conformation of the ring and the dissymetric environment of the chromophore. A rule proposed by Snatzke et al. [11] for steroidal cyclic azomethines [11] has been used successfully for a number of alkaloids with azomethine rings [12]. According to this rule, an azomethine chromophore possesses *a* positive EC in the *a* conformation of the Δ^1-piperidine ring and a negative one in the b conformation (Fig. 2). This rule has a formal similarity to the octant rule for the carbonyl group [10] and was first established by Djerassi and Klyne [13] for *trans*-decalone and cyclohexane systems in the *twist* conformation. As mentioned in [11], the molecular orbitals of the C=N and C=O bonds in an azomethine group and a keto group differ in their symmetry. Because of the absence of symmetry of the π-orbital of the azomethine bond relative to the vertical X,Y plane, the space around the chromophore is divided by curved nodal surfaces of the orbitals into "octants" not symmetrical with respect to one another, which complicates the application of the well-known octant rule to azomethines for predicting the sign of the CE in the general case.

* The numbering of the atoms of the isoxazoline ring corresponds to the numbering of the side-chain of cholesterol.

Institute of Bioorganic Chemistry, Belarus Academy of Sciences, Minsk. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 391-397, May-June, 1994. Original article submitted May 4, 1993.

0009-3130/94/3003-0357$12.50 ©1995 Plenum Publishing Corporation

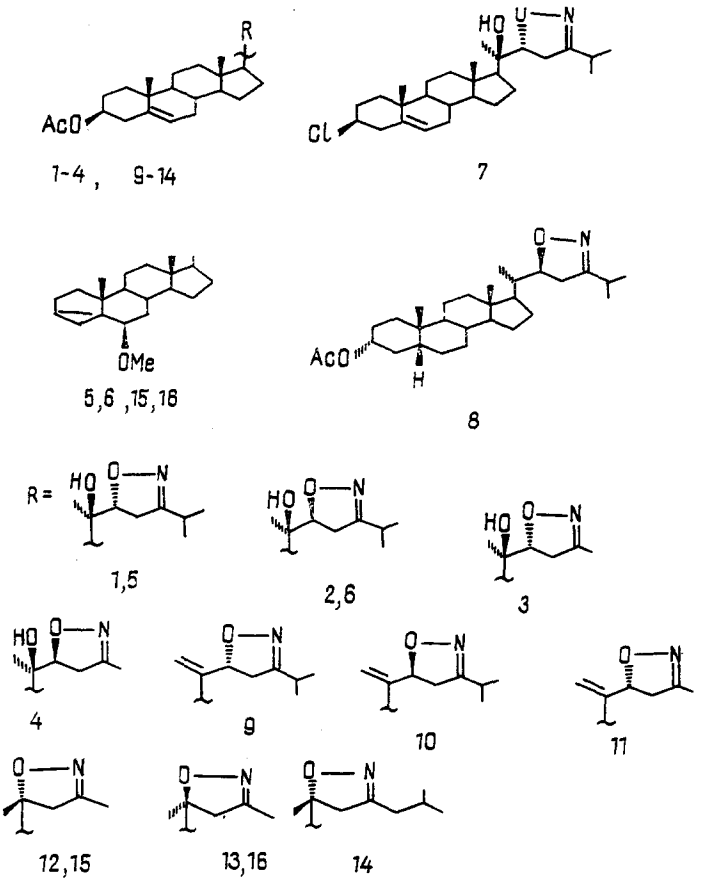

TABLE 1

Compound	λ (ε)	λ ([Θ])
	20-Isoxazolinylsteroids	
1	220 shoulder	212 (—30)
2	220 shoulder	215 (+30)
3	223 shoulder	212 (—30)
4	224 shoulder	215 (+18)
5	215 shoulder	212 (—30)
6	214 shoulder	214 (+20)
7	212 shoulder	215 (—27. 295 (—5)
8	215 shoulder	215 (+20)
9	217 shoulder	215 (—35)
10	220 shoulder	220 (+7.5)
11	217 shoulder	215 (—38)
	17-Isoxazolinylsteroids	
12	213 shoulder	213 (—35)
13	216 shoulder	216 (+17)
14	216 shoulder	216 (—28)
15	214 shoulder	212 (—27)
16	213 (3900)	214 (+22)

However, we have attempted, with some circumspection, to use the octant rule for describing the CD of cyclic steroidal azomethines, taking into account the basic contribution of the atoms of the azomethine ring to the sign and magnitude of the EC.

The geometry of the five-membered isoxazoline ring differs substantially from that of the six-membered Δ^1-piperidine ring. The presence of two neighboring heteroatoms and a sp^2-hybridized carbon leads to a pronounced flattening of the ring, which permits an only slight departure of the C-22 atom from the plane of the ring. Compounds with isoxazoline and Δ^1-

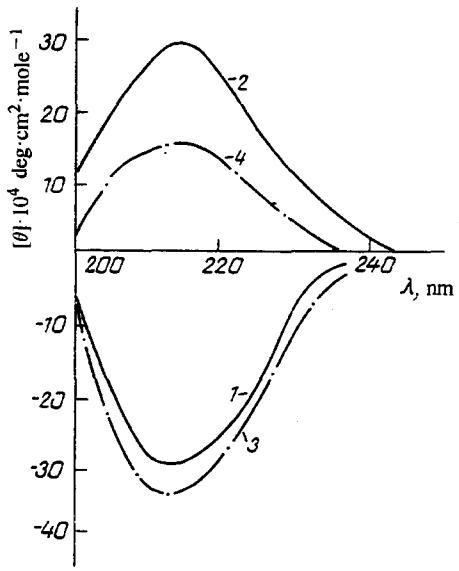

Fig. 1. CD spectra of the 20-isoxazolinylsteroids (**1**) (1) and (**2**) (2) and of the 17-isoxazolinylsteroids (**12**) (3) and (**13**) (4).

Fig. 2. *a* and *b* conformations of the Δ^1-piperidine ring.

piperidine rings also differ in their spectral properties. For compounds with a Δ^1-piperidine ring the $n-\pi^*$ transition of the azomethine chromophore is detected in the form of a weak band at about 250 nm [11]. In the absorption spectra of the compounds that we have considered, the band of the $n-\pi^*$ transition of this chromophore in the isoxazoline ring was shifted into the short-wave region (212-220 nm) and was observed, as a rule, in the form of a shoulder on the long-wave wing of the band of the more intense $\pi-\pi^*$ transition. We were unable to observe any other long-wave band, either in the absorption spectra or in the CD spectra, except for compound (**7**), for which a small negative CD band was observed at 295 nm the origin of which will be given below.

The application of the octant rule to the azomethine bond of the 20-isoxazolinylsteroids (**1-7**) with the use of Dreiding molecular models permits the projections of the compounds illustrated in Fig. 3 to be obtained. The negative sign of the 212-nm CE of epimer (**1**) with the previously established R configuration of the C-22 asymmetric center is determined, above all, by the position of the C-22 carbon atom with a proton in the negative right upper octant, in spite of the fact that a considerably greater number of atomic groups belonging mainly to the steroid skeleton falls into the positive right lower octant (Fig. 3a).

The remaining atoms of the isoxazoline ring are present in the plane of the chromophore and make no contribution to the optical rotation. The remote skeletal atoms, the distribution of which over the octants may not be strict because of the above-mentioned distortion of the nodal octant surfaces, affect the magnitude of the CE only feebly. A more substantial influence on the optical activity of the chromophore should apparently be exerted by the atoms of the isopropyl radical at C-24, which are linked directly to the azomethine group, and are located in the front left octants. However, the role of this radical and other substituents at C-24 can be elucidated by comparing the magnitudes of the CEs in the series of compounds considered. Analogously, from an analysis of the distribution of the atomic groups of the epimer (**2**) with the S configuration of the C-22 center in the octants (Fig. 3b) it may be concluded that the predominating contribution to the positive sign of the 215-nm CE is made by the C-22 atomic group of the ring.

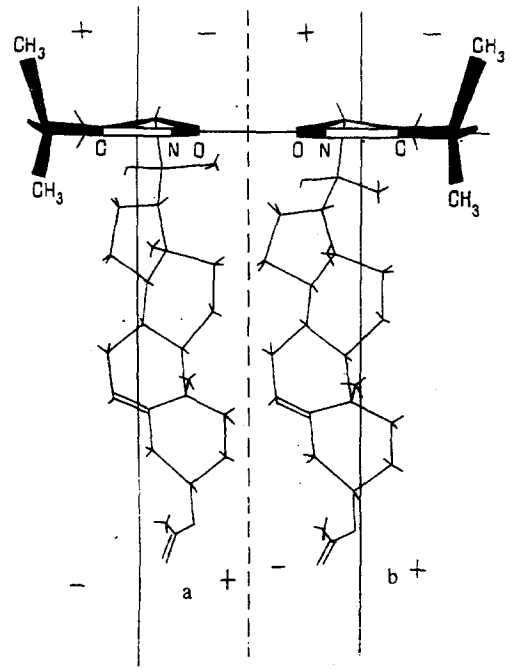

Fig. 3. Projections of the 20-isoxazolinylsteroid (**1**) (a) and its epimer (**2**) (b) onto the rear octants.

The stereochemical assignments made for the pair of epimers (**1**) and (**2**) can be extended to other isomeric pairs or individual spatial isomers in the series of 20-isoxazolinylsteroids (**3-11**) and permit compounds with a negative CE in the 212-220 nm region to be ascribed the 22R configuration of the active center, and compounds with a positive CE the 22S configuration. In addition, we have attempted to find definite structural relationships from a comparison of the absolute values of the molecular ellipticities in the CD spectra of the 20-isoxazolinylsteroids (**1-11**), although the low accuracy of the recording of optical rotation in the 200-220 nm region gives no grounds for strict conclusions.

It must be mentioned that the epimers (**1**, **3**, and **5**) with the 22R configuration of the asymmetric center have the same molecular ellipticity of their CD bands (Table 1). The somewhat lower value of the ellipticity for the epimer (**7**) in the CD spectrum of which an additional weak band is observed at 295 nm is most probably due to to the presence of a small amount of a 3-ketosteroid in the sample investigated. On the basis of the octant rule, the identical values of the molecular ellipticities in the epimers (**1**, **3**, **5**, and **7**) must be explained by identical contributions of methyl and isopropyl radicals at C-24 to the optical activity of the chromophore, which is possible if the radicals rotate freely around the C-24–C-25 bond. As can be seen from a consideration of molecular models, such a possibility is likewise realised in the epimers with the 22R and 22S configurations of the asymmetric center. However, the molecular ellipticities of the epimers (**2**, **4**, and **6**) with the 22S configuration not only differ from one another but also from those of the corresponding 22R-epimers (with the exclusion of epimer (**2**)). These differences are apparently connected not with structural features of the epimers but with a concentration effect. In our opinion, the lower values of the molecular ellipticities for the 22S-epimers than for the 22R-epimers were due to the presence of optically inactive impurities in the specimens analyzed.

In a comparison of the absolute values of molecular ellipticities one must also take into consideration the presence of additional chromophores in the structures of individual compounds. The contribution of a Δ^5 bond in ring B of the steroid skeleton to the optical rotation must be insignificant, since the maximum of the $\pi-\pi^*$ transition in this bond is located at about 195 nm [14]. We detected no appreciable changes whatever in the CD of the 20-isoxazolinylsteroids (**1-7**) that could be connected with the influence of the Δ^5 bond. However, it may be concluded from a comparison of the magnitudes of the molecular ellipticities of the 17-isoxazolinylsteroids (**12**) and (**15**), and (**13**) and (**16**), that this bond makes some negative contribution to the CE.

At the same time, one must expect a more substantial contribution of the $C_{20}=C_{21}$ bond to the optical activities of the 20-isoxazolinylsteroids (**9-11**), the band of the $\pi-\pi^*$ transition in which falls into the 200-220 nm region [10, 14] and overlaps with the band of the azomethine chromophore. The application of the octant rule to the main $\pi_x-\pi_x^*$ transition of the $C_{20}=C_{21}$ bond [10] leads to a negative CE, since the whole steroid skeleton of the molecule falls into the negative right upper octant, while only the isoxazoline ring falls into the positive left upper octant of the rear side of the octants. This contribution of the side-chain double bond to the optical rotation substantially changes the ellipticities of compounds (**9-11**) in comparison with the corresponding epimers in the series of 20-isoxazolinylsteroids (**1-8**) (see Table 1).

It must also be mentioned that the 20-isoxazolinylsteroids (**9-11**) differ substantially in the chemical respect from the compounds (**1-8**) considered above. The presence of the rigid C-20=C-21 fragment leads to an increase in the spatial interactions involving the protons of the double bond and of the isoxazoline ring with the protons at the C-19, C-12, and C-16 atoms. The molecular projections on the rear octants of epimers (**9-11**) have a form somewhat different from those given in Fig. 3. The majority of the atoms of the steroid skeletons in the 22R-epimers (**9**) and (**11**) fall into the negative left lower octant, while only the C-20, C-21, C-12, and C-11 atomic groups fall into the positive right lower octant, which must lead to some increase in molecular ellipticity for these epimers.

For the 22S-epimer (**10**) we observed an anomalously low ellipticity, and this, in our view, is also explained by its geometry. As can be seen from a consideration of molecular models, apart from the atoms of the isoxazoline ring, all the atoms of the the molecule, without exception, fall into the negative left lower octant, which causes a a pronounced decrease in the positive CE.

We have also applied the octant rule to the 17-isoxazolinylsteroids (**12-16**). As in the 20-isoxazolinylsteroids with a double bond in the side-chain, in these compounds there is a strong intramolecular interaction of the closely adjacent C-19 and C-21 methyl groups and also of the protons at C-16, C-12, and C-22, which limits the configurational mobility of the side-chain, particularly in the epimers with the 20R-configuration of the asymmetric center. The 17-isoxazolinylsteroids differ greatly from the 20-isoxazolinylsteroids by the positions of the atoms of the steroid skeletons in molecular projections. Thus, in the 20R-epimers (**12**, **14**, and **15**) all the atoms of the steroid skeleton, with the exception of C-16, are in the positive right lower octant, while the C-21 methyl group and the C-16 methylene group are in the negative right upper and left lower octants. In the case of the 20S-epimers (**13**) and (**16**), all the atoms of the steroid skeleton fall into the negative left lower octant, and only the C-21 methyl group is located in the positive left upper octant.

As can be seen from a comparison of the absolute values of the molecular ellipticities of the 17-isoxazolinylsteroids (**12-16**) (see Table 1), the sign of the CD band is determined mainly by the conformation of the isoxazoline ring. However, it is impossible not to consider the influence of remote groups of the steroid skeleton and, in particular, the closely positioned C-21 methyl on the magnitude of the CE. It is just because of the positive contribution of this methyl group that no anomalously low values of the ellipticity are observed in the 20S-epimers (**12** and **16**) as is the case for the 22S-epimer of the 20-isoxazolinylsteroid (**10**). Similarly, the negative contribution of the C-21 methyl group to the optical rotation of the 20R-epimers (**12**, **14**, and **15**) does not permit an appreciable change in the magnitude of the CE in comparison with the corresponding epimeric 20-isoxazolinylsteroids because of the opposite influence of the atoms of the steroid skeleton.

EXPERIMENTAL

The epimers were separated on a column of SiO_2, using a mixture of hexane and ether as the eluent. The ratios of the epimers isolated were 3-6:1 for the different types of isoxazolinylsteroids.

The CD spectra of the compounds under investigation were recorded on a JASCO-20 spectropolarimeter in ethanolic solution at concentrations of $4\cdot10^{-4}$ to $1\cdot10^{-3}$ M in quartz cells 0.2 and 0.5 cm thick. The sensitivity of the instrument was 0.005°/cm, the time constant 4, and the rate of scanning 1 nm/min. Molecular ellipticity was determined on the basis of three measurements, and the relative error did not exceed 20%. Absorption spectra were measured on Specord UV VIS and Specord M-400 spectrophotometers at the same concentrations and cell thicknesses. To reveal the possible presence of additional bands in the long-wave regions of the absorption and CD spectra we used cells 2 cm thick.

REFERENCES

1. A. A. Akhrem, V. A. Khripach, R. P. Litvinovskaya, A. V. Baranovskii, et al., Zh. Org. Khim., **25**, No. 9, 1901 (1989).

2. V. A. Khripach, R. P. Litvinovskaya, A. V. Baranovskii, and E. A. Ermolenko, Khim. Geterotsikl. Soedin., No. 10, 1389 (1990).
3. A. I. Verenich, A. A. Govorova, N. M. Galitskii, A. V. Baranovskii, et al., Zh. Strukt. Khim., No. 5, 152 (1992).
4. V. A. Khripach, R. P. Litvinovskaya, and A. V. Baranovskii, Mend. Commun., No. 3, 117 (1992).
5. V. A. Khripach, R. P. Litvinovskaya, and A. V. Baranovskii, Dokl. Akad. Nauk SSSR, **318**, 597 (1991).
6. V. A. Khripach, R. P. Litvinovskaya, A. V. Baranovskii, and S. V. Drach, Zh. Org. Khim., **29**, 1224 (1993).
7. V. A. Khripach, R. P. Litvinovskaya, and A. V. Baranovskii, in: XVIIth IUPAC International Symposium on the Chemistry of Natural Products, New Delhi (1990), p. 222.
8. V. A. Khripach, R. P. Litvinovskaya, and A. V Baranovskii, Khim. Prir. Soedin., 143 (1992).
9. A. V. Baranovskii, R. P. Litvinovskaya, and V. A. Khripach, Zh. Org. Khim., **26**, No. 6, 1274 (1990).
10. P. Crabbé, An Introduction to the Chiroptical Methods in Chemistry, Offsali, Mexico (1971).
11. H. Ripperger, K. Schreiber, and G. Snatzke, Tetrahedron, **21**, No., 5, 1027 (1965).
12. G. P. Moiseeva, A. Nabiev, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 345 (1986).
13. C. Djerassi and W. Klyne, Proc. Natl. Acad. Sci. USA, **48**, No. 6, 1093 (1962).
14. B. A. Kazanskii, Reactions and Methods of Investigating Organic Compounds, Book 18 [in Russian], Khimiya, Moscow (1967).