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The Study of UV and CD Spectra of D-Arabino-hexulosephenylosazone and D-Arabino-hexulose-2,4-dinitrophenylosazone in Various Solvents

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The ultraviolet (UV) and circular dichroizm (CD) spectra of D-arabino-hexulosephenylosazone (*PH*) and D-arabino-hexulose-2,4-dinitrophenylosazone (*DNPH*) in various solvents were studied. The experimentally obtained curves were resolved into single bands according to non-linear least-squares methods. The quantity of bands, the positions of their absorption maxima, half-band width oscillator strength and rotator strength were determined. Application of the CD method allowed to identify the lowest excited electronic state of *PH* osazone.

(Keywords: Circular dichroism; Osazones; UV, osazones)

Analyse der UV und CD Spektren von D-Arabino-hexulosephenylosazon und D-Arabino-hexulose-2,4-dinitrophenylosazon für verschiedene Lösungsmittel

Ultraviolett (UV) und Circulardichroismus (CD) der Titelverbindungen wurden mittels einer nichtlinearen-kleinsten-Fehlerquadratmethode in die einzelnen Banden aufgelöst. Wellenlänge, Bandbreite und Oszillatoren- bzw. Rotatorstärken wurden für verschiedene Lösungsmittel bestimmt und diskutiert.

Introduction

Although the first osazones of carbohydrates were obtained in 1884 by *E. Fischer*[1], up to now—as far as we know—their spectral, photochemical and photophysical properties have not been studied in detail[2]. However, there are some papers which have been concerned with the determination of the structure of osazones and with their

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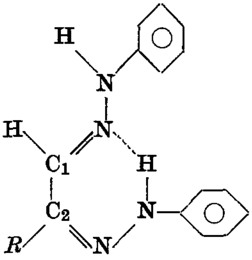
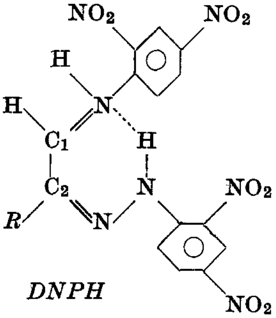
reactions. The structures of both osazones, according to *Mester* and *El-Khadem*[2,8,9], are shown below:



PH



DNPH

where R is $(CHOH)_3CH_2OH$.

The UV and CD spectra were studied only qualitatively for a few of carbohydrates[3–11]. There is no data in the literature about the UV spectra of *DNPH* osazone and about the CD spectra of both osazones investigated, as well as any attempts to resolve these and other carbohydrates spectra to simple bands. Therefore, this paper is concerned with the UV and CD spetra of *PH* and *DNPH* osazones, with the resolving of these spectra to the single bands and with their quantitative description.

Results

The UV and CD spectra were taken in dimethylformamide (*DMF*), acetonitrile, ethanol, dioxane and benzene in concentration range $5.6 \cdot 10^{-3}$–$5.6 \cdot 10^{-6}$ M for *PH* osazone and $3.75 \cdot 10^{-3}$–$3.75 \cdot 10^{-6}$ M for *DNPH* osazone, respectively. In order to avoid mutarotation, all spectra were taken immediately after preparing the solutions. The solvents used were chosen because of their different polarity, dielectric constant, dipole moment, donor number, basicity constant and the possilibity of creating hydrogen bonds. Thus, the identification of the possibly overlapping absorption bands and the description of electronic states of the molecule connected with these bands were possible.

All the UV and CD spectra of *PH* osazone in various solvents are similar to each other[9] (the CD spectrum in acetonitrile is an exception). In the case of UV and CD spectra of *DNPH* osazone a shift of more then 1,500 cm^{-1} of long-wavelength bands absorption maxima was found to occur depending on the solvent applied (Fig. 1, Tab. 2—bands 4, 5 and 6).

Table 1. *Parameters of bands in the UV spectrum of PH osazone in various solvent*

Solvent					bands			
		1	2	3	4	5	6	7
CH_3CN	a	49,500*	43,730	40,030	38,560	32,220	25,890	24,540
	b	—	0,352	0.131	0.065	0.340	0.228	0.050
	c	—	6,310	3,190	2,290	7,740	3,640	2,100
Ethanol	a	48,730	43,770	39,600	38,110	31,810	25,970	24,200
	b	0.169	0.444	0.140	0.068	0.354	0.316	0.046
	c	2,820	7,350	3,200	2,150	7,380	3,790	1,970
Dioxane	a			39,950	38,220	32,000	25,600	24,260
	b	nobs	nobs	0.185	0.084	0.353	0.244	0.041
				3,260	2,180	7,720	3,610	1,950
DMF	a					31,950	25,590	24,280
	b	nobs	nobs	nobs	nobs	0.278	0.245	0.025
	c					6,710	3,740	1,720

a—Position of absorption maximum (cm^{-1}); b—oscillator strength (dimensionless); c—half-band width (cm^{-1}); nobs—non-observed band because of solvent absorption; *—value taken from experimentally measured spectrum.

Table 2. *Parameters of bands in the UV spectrum of DNPH osazone in various solvents (for indices see Tab. 1)*

Solvent				bands			
		1	2	3	4	5	6
CH_3CN	a	46,850*	38,700	32,920	29,550	25,030	21,480
	b	—	0.552	0.040	0.193	0.669	0.043
	c	9,500*	7,090	2,620	4,870	6,290	2,370
Ethanol	a	46,170	38,020	33,070	29,210	24,700	21,730
	b	0.852	0.306	0.032	0.232	0.524	0.081
	c	10,000	5,910	2,510	5,340	5,480	2,580
Dioxane	a		37,610	33,020	28,520	25,080	21,820
	b	nobs	0.124	0.011	0.273	0.420	0.173
	c		3,910	2,250	5,200	4,410	2,850
DMF	a			32,480	27,600	24,010	21,380
	b	nobs	nobs	0.013	0.303	0.532	0.110
	c			1,950	4,670	4,580	2,580
Benzene	a			32,900	28,480	25,790	22,660
	b	nobs	nobs	0.013	0.288	0.113	0.185
	c			2,410	5,370	3,290	5,520

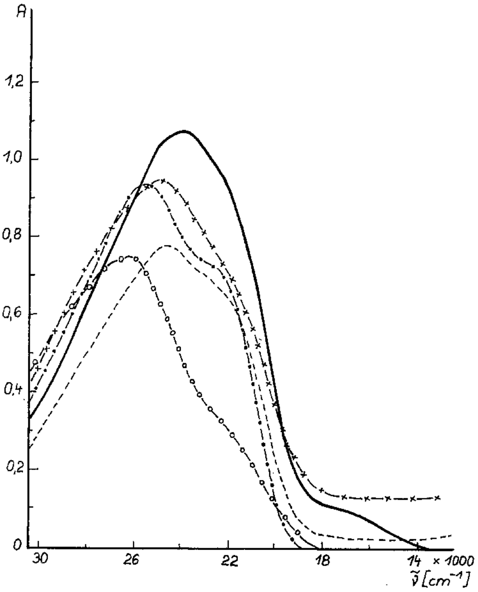


Fig. 1. UV spectrum of *DNPH* osazone in various solvents: ——— *DMF*, - - - - ethanol, —·—·— dioxane, —×—×— acetonitrile, —○—○— benzene

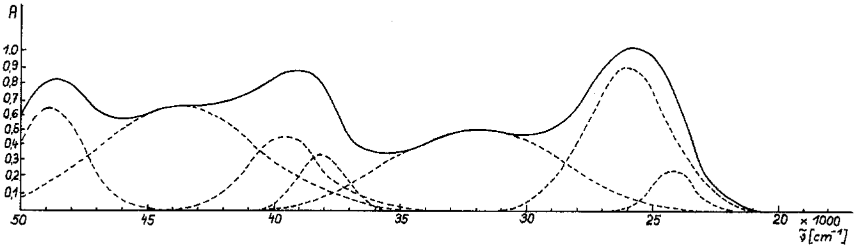


Fig. 2. UV spectrum of *PH* osazone in ethanol: ——— experimental, - - - - resolved

Typical UV and CD spectra of both osazones in 95% ethanol, resolved into the single bands, are shown in Figs. 2–5.

Each of the absorption band was described by the *Gauss*ian function:

$$A_G(\nu) = x_1 \exp\left[-x_3^2 (\nu - x_2)^2\right]$$

where $A_G(\nu)$ is the absorption at the frequency ν, x_1, x_2, x_3 are simply connected to the value of absorption maximum, its position and half-band width of the band, respectively. The convergence between the

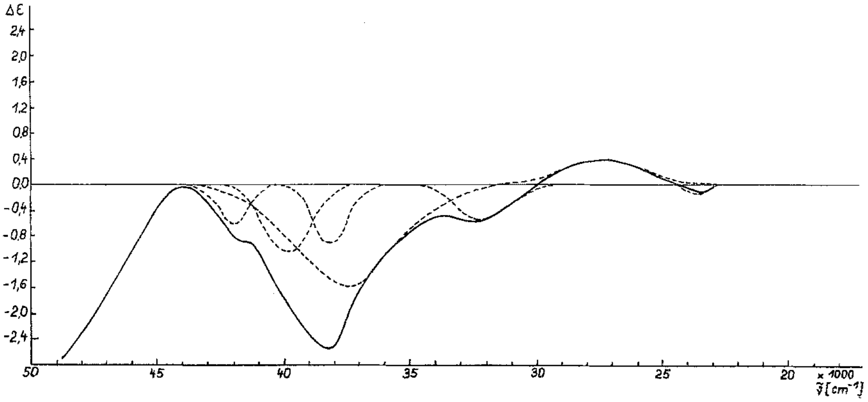


Fig. 3. CD spectum of *PH* osazone in ethanol: ——— experimental, - - - - resolved

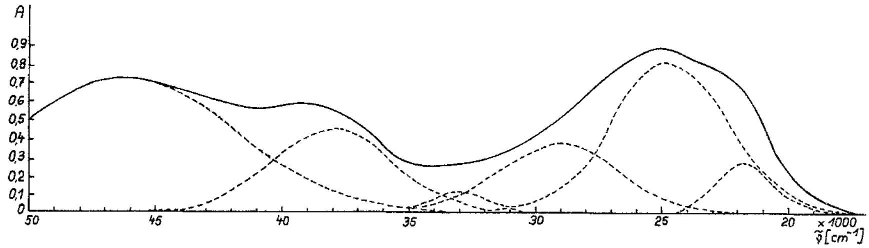


Fig. 4. UV spectrum of *DNPH* osazone in ethanol: ——— experimental, - - - - resolved

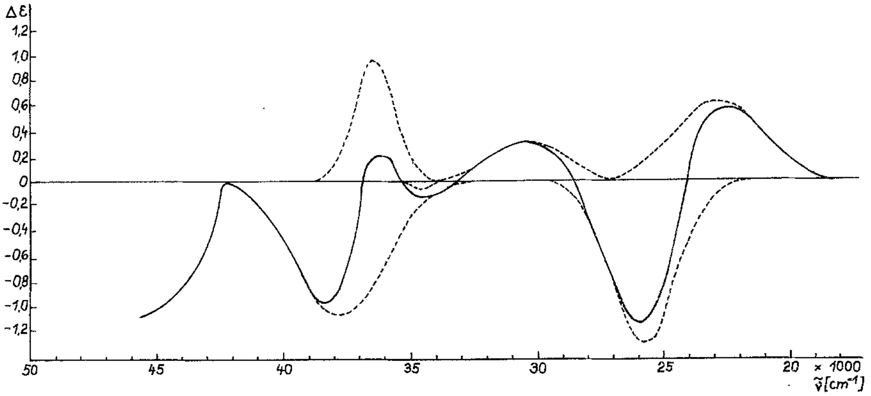


Fig. 5. CD spectrum of *DNPH* osazone in ethanol: ——— experimental, - - - - resolved

observed and calculated absorption spectra was achieved by means of the least squares method, minimizing the error function Φ:

$$\Phi = \sum_{i=1}^{M} (A_i^{\text{obs}} - A_i^{\text{cal}})^2$$

where A_i are the absorption values, observed (obs) and calculated (cal), and M is a number of measurement points. The *Meiron* method[12, 13] was applied in order to determine the x parameters giving the minimum value of the error function. Two criteria were applied in order to estimate the accuracy and stability of final results:

i. the standard error S, $S = \{\Phi/M - (3N + 2)]\}^{1/2}$ where N is the number of bands;

ii. the norm of gradient of the error function Φ.

In all cases the observed spectra were resolved possibly into the least number of bands, in order to obtain the best convergence between the observed and calculated spectra. The calculations were ended when the norm of gradient of the error function was smaller than 0.01. In all cases, good agreement was achieved between the observed and calculated spectra. The mean value of the standard error S for the resolved spectra was 0.0081 of the absorption units. The values of oscillator strength f and rotator strength R were calculated according to the formulas:

$$f = 4.32 \cdot 10^{-9} \int \varepsilon(\nu)\, d\nu \text{ (dimensionless)}$$

$$R = 0.248 \int [\Delta\varepsilon(\nu)/\nu]\, d\nu \text{ (\textit{Debye-Bohr} magneton)}$$

where $\varepsilon(\nu)$ is a molar absorption coefficient and $\Delta\varepsilon(\nu)$ is a circular dichroism for the frequency ν.

The quantitative data of UV spectra resolved into single bands for both the osazones in various solvents are shown in Tab. 1 and 2.

The parameters of the bands in CD spectrum in 95% ethanol for both the osazones are listed in Tab. 3.

For both osazones with the expection of *DNPH* in *DMF* the UV spectra did not show the deviation from the *Lambert-Beer*'s law, in the whole range of concentrations. In *DMF*, however, the long-wavelength band with the absorption maximum at $\nu = 18{,}250\,\text{cm}^{-1}$, $\varepsilon_{18,250} = 42{,}000$, half-band width $7{,}820\,\text{cm}^{-1}$ and $f = 1.510$, appeared. This band originates probably from the anion form of osazone and is distinctly seen both in the UV spectrum and in the CD spectrum. The intensity of the band is changing with the osazone concentration due to the change in concentration of the anion form of osazone with the dilution of the solution (Fig.6). This phenomen was not observed in any other solvent.

Table 3. *Parameters of bands in the CD spectrum of DNPH osazone and PH osazone in 95% ethanol*

Osazone		bands 1	2	3	4	5	6	7	8
DNPH	a	46,500	37,800	36,430	34,410	30,270	25,740	22,710	
	b		—0.031	0.014	—0.0003	0.009	—0.041	0.031	
	c	2,750	4,060	1,890	650	3,160	2,960	4,080	
PH	a	48,700	42,040	39,840	38,230	37,530	32,190	27,380	23,670
	b		—0.005	—0.013	—0.009	—0.047	—0.009	—0.014	—0.002
	c	2,800	1,450	2,120	1,630	5,060	2,450	3,980	1,120

a—Position of absorption maximum (cm^{-1}); b—rotator strength (*Debey-Bohr* magnetone); c—half-band width (cm^{-1}).

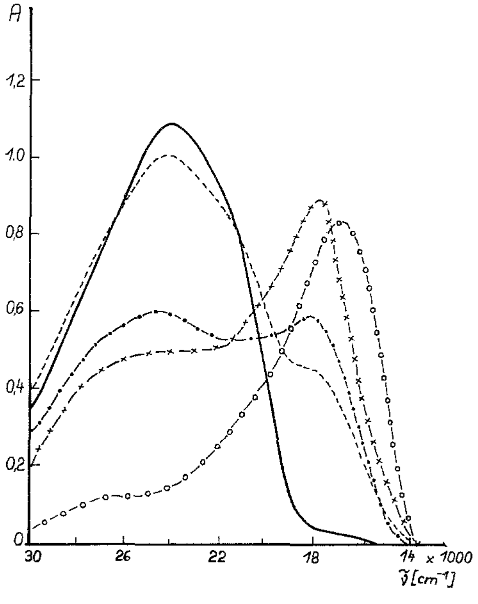


Fig. 6. UV spectrum of *DNPH* osazone in *DMF* for various osazone concentrations: ——— $3.75 \cdot 10^{-4}$ *M* (0.1 cm), ---- $3.75 \cdot 10^{-5}$ *M* (1 cm), -·-·- $1.4 \cdot 10^{-5}$ *M* (2 cm), —×—×— $0.75 \cdot 10^{-5}$ *M* (5 cm), —○—○— $0.45 \cdot 10^{-5}$ *M* (4 cm); in parenthesis path length is given

Discussion

The UV spectrum of *PH* osazone is relatively complex. Because of the great intensities of the observed absorption bands ($\varepsilon > 5 \cdot 10^3$) and the lack of the clear shifts of the bands in *PH* osazone spectra with the change of solvent properties (Tab. 1), only π, π^* and intramolecular charge-transfer bands can be observed. Therefore, the expected n, π^* bands, are not observed.

To date various authors[3–5,9] were concerned only with the bands which are clearly seen in UV at 38,900, 32,600 and 25,150 cm^{-1}. Our results, however, show for *PH* osazone seven absorption bands, five of them seen in the experimentally measured UV spectrum (Fig. 2). The sixth absorption band of the longest wavelength and of the relatively weakest intensity (Tab. 1—band 7) can be observed in the CD spectrum, maximum at ν = 23,670 cm^{-1} (Tab. 3—band 8), although it is not directly observed in the UV spectrum. The presence of this band was confirmed independently in the investigation of photomutarotation and photolysis of *PH* osazone[14]. The seventh and eighth absorption bands (Tab. 1—band 4, and Tab. 3—bands 3, 4), neither in UV nor in CD spectra can be observed so clearly. Yet, the best convergence between the observed and resolved into the single bands spectra, both the UV and CD, was achieved by taking into account the presence of an additional band just in this absorption range.

The CD spectrum of *PH* osazone is also relatively complex (Fig. 3) and six absorption bands can be distinctly observed.

Comparison of UV and CD bands of *PH* osazone gives fairly good accordance of wavelengths of absorption maxima for both spectra. Relatively small deviations can be possibly connected with the necessity of the recording of CD spectra at large ratio of noise to the signal, because of the very small $\Delta\varepsilon$ values for the majority of bands. Small values of $\Delta\varepsilon$ and R confirm that the observed transitions in CD spectrum are magnetic forbidden and they can be observed probably because of the vibronic coupling. In this case total accordance in position and intensity distribution of UV and CD bands cannot be expected.

The solvent effect on UV and CD spectra of *DNPH* osazone is of a different nature than in the case of *PH* osazone because of the presence of four nitro groups in the molecule having strong electron-acceptor properties. This is demonstrated particulary by the positions of the long-wavelength absorption bands in visible region (Fig. 4) which are shown in Tab. 2. It is obvious, on the basis of the obtained result, that they are the π, π^* bands eventually with the various contribution of intramolecular charge-transfer.

Both the UV and CD spectra of *DNPH* and *PH* osazones in the same solvent differ strongly (Tab. 1-3, Figs. 2-5). In the UV spectrum, absorption maxima in the case of *DNPH* are bathochromically shifted and the molar absorption coefficients for the particular bands increase (Figs. 2, 4). The similar effect can be observed in the CD spectrum of both osazones (Figs. 3, 5). Among the other factors, this may be connected with the structure of both the osazones and properties of nitro groups enabling the considerable increase of π electron coupling. The molecule of *PH* osazone with the exception of the sugar rest, and the majority of the molecule of *DNPH* osazone are flat. Both, in the *PH* and, probably, in the *DNPH* osazone molecules intramolecular hydrogen bonding exists, enabling the creation of an N-chelate structure[8, 15–19]. This is the reason why the molecules exhibits nonclassic[20, 21] aromatic properties. Besides, in *DNPH* osazone at least two additional hydrogen bondings exist.

Table 4. *Physicochemical properties of solvents used*

Solvent	ε	μ	E_T	P	Y	$pK\frac{\text{base}}{H_2O}$
DMF	37	3.86	43.3	11.5	30.9	0.0
CH_3CN	37	3.44	46	11.8	14.1	—4.4
Ethanol	24	1.69	51.9	11.2	—	—2.4
Dioxane	2.2	0.45	36	9.8	—	—4.4
Benzene	2.3	0.0	34.5	9.2	—	—14.8

ε—Dielectric constant; μ—dipole moment (*Debyes*); E_T—empirical polarity measure of solvent according to *Dimroth*[27]; P—solubility parameter of *Hildebrandt*[28]; Y—donor number[29]; $pK\frac{\text{base}}{H_2O}$—basicity constant[30].

The UV spectra of *DNPH* and 2,4-dinitrophenylhydrazones[22–24] were compared in order to estimate the interaction between two 2,4-dinitrophenylhydrazone groups in the *DNPH* osazone molecule[22–24]. This comparison shows that the absorption bands of *DNPH* osazone are bathochromically shifted. This confirms the additional stabilization of the osazone molecule because of both, the presence of hydrogen bonding, enabling the creation of an N-chelate structure, and the delocalization of π-electrons over a greater number of atoms.

When *DMF* was applied as a solvent, considerable changes in UV and CD of *DNPH* osazone occured. The long-wavelength band with large intensity and half-band width appeared in both spectra. The intensity and half-width of this band strongly depend on the osazone concentration (Fig. 6). Although the dielectric constant and most of the other quantities described the polar character of *DMF* (Tab. 4) are very similar to those of acetonitrile and ethanol, the basicity constant and donor number are clearly the biggest ones in *DMF*. Thus, it seems, that this band originating from the anionic form of osazone, is formed as a result of proton dissociation from nitrogen atom at C-2. This was also confirmed by UV spectra in acidic and basic media. The appearance of anion form absorption in the long-wavelength absorption range is a result of a considerable increase of the delocalization of π-electrons over the whole molecule (with the exception of the sugar rest) and is in agreement with the literature data of UV spectra for anion forms of 2,4-dinitrophenylhydrazones[22–24].

The values of energies of the excited states obtained from analysis of UV and CD spectra were used in the investigation of photomutarotation and photolysis of both *DNPH* and *PH* osazones[14].

Experimental

Osazones were obtained according to *Neuberg* and *Noyen*[25,26]. The osazones were crystallized repeatedly from water and ethanol. The purity of *DNPH* and *PH* was checked by measurements of melting points, by elementary analyses and by IR applying Perkin Elmer's elementar analyser Model 240 and UR-20 and Specord IR 71 C. Zeiss spectrophotometers. The obtained results were in agreement with the literature data.

Special for fluorescence and spectrally pure solvents (Merck and Fluka) were additionally dried. The UV spectra were taken on UNICAM SP-700, Carl Zeiss Specord and VSU-2 spectrophotometers. The mercury lamp, $NdCl_3$, $PrCl_3$, $SnCl_3$ and neutral filters (with exactly determined absorption for different wavelenghts) were applied as calibration standards of wavelength and absorption of spectrophotometers. The measuring error of wavelength $\Delta\lambda$ was $\Delta\lambda \leq 1$ nm for $\lambda < 350$ nm and $\Delta\lambda \leq 2$ nm for $350 < \lambda < 800$ nm. The error of the absorption values was less than 3%. The CD spectra were carried out on Jobin Yvon MarcIII dichrograph with the accuracy $\Delta\varepsilon = 0.01$ and $\Delta\lambda \leq 1$ nm. The concentration of solutions and the path length of the cell used for the UV and CD spectra measurements, were selected to ensure the absorption in the range $0.2 < A < 1$. Odra 1204 computer was applied to calculate the bands distribution in the spectra.

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