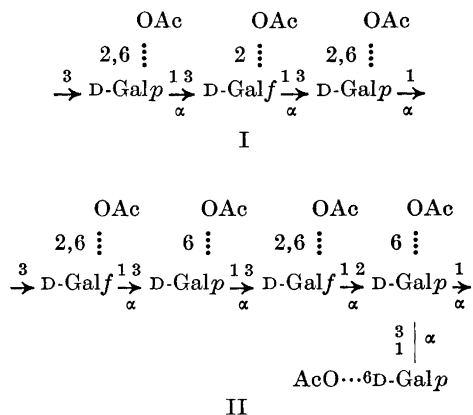


by Kaluzewski⁸ that they are serologically identical is, however, difficult to understand in the light of the present results.



Experimental. General methods and methods for sugar analysis, methylation analysis, and location of *O*-acetyl groups were essentially the same as used in studies on the related *Klebsiella* O group 9 LPS.³ GLC of the partially methylated alditol acetates was performed at 220° on a glass capillary column (0.25 mm × 25 m) containing SP-1000 as the stationary phase. For GLC-MS an OV-225 SCOT column was used. The LPS was isolated¹ from strain *Klebsiella* O8:K69 (889), and showed $[\alpha]_{578} + 59^\circ$ (*c* 0.25, water). In the IR (KBr) a strong absorption at 1730 cm⁻¹ was observed. The LPS contained 1.8 % P.

Analysis of partially hydrolysed LPS. The LPS (5 mg) in 0.025 M sulphuric acid (5 ml) was kept at 100° for 2 h and neutralised (BaCO₃). Sodium borodeuteride (20 mg) was added and the solution kept for 3 h at room temperature. The solution was treated with Dowex 50 (H⁺), concentrated and boric acid removed by distillations with methanol. The product was dissolved in water, lyophilised and subjected to methylation analysis.

Periodate oxidation of the LPS. The LPS (5 mg) was dissolved in 0.1 M sodium acetate buffer of pH 3.9 (5 ml), 0.08 M sodium metaperiodate (2 ml) was added and the solution kept in the dark at 4° for 48 h. Excess periodate was reduced with ethylene glycol (0.5 ml) and the solution was dialysed against tap-water overnight. Sodium borohydride (60 mg) was added, the solution kept for 7 h at room temperature, excess borohydride decomposed by addition of 50 % acetic acid and the solution dialysed overnight. Sugar analysis of this

product showed arabinose and galactose in the molar proportion 1:1.9. D-Mannose (1 mg) was added as an internal standard, and the two sugars accounted for all the galactose in the original LPS.

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On the Calibration of Circular Dichroism Spectrometers

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Due to uncertainties about CD standards¹⁻³ we want to suggest two methods of calibrating a CD spectrometer. The first determines whether the instrument is actually measuring $CD = A_1 - A_2$. It may also be used for the calibration over a wavelength range, in which the instrumental accuracy depends upon the wavelength program for the excitation voltage of the light modulation.⁴ The second is appropriate for regular checking.

The normal calibration methods are subject to the following uncertainties: 1. They require the use of a "standard" sample of previously established specific CD. This $\Delta\epsilon$ may be inaccurate. 2. The standard may be of poor optical or chemical purity. 3. The chemical and instrumental conditions to which the $\Delta\epsilon$ refers may be poorly defined. As none of the commercially available CD instruments yields an absolute CD, the first point is the most intractable.⁴⁻⁶

The first method does not require any optically active sample. Hence it does not rely upon a previously established specific CD. Instead it takes advantage of the fact that the accuracy of the optical decadic absorbance obtained from a spectrophotometer, $\log(I_0/I)$, can be determined easily.⁷ A Glan polarizing prism was placed in a Zeiss PMQ-II spectrophotometer. The two orthogonal polarizations, A_{\parallel} and A_{\perp} ,⁸ of a stretched polyethylene film containing orientated 2,2'-bipyridyl were measured at 282 nm where the wavelength of the broad absorbance maximum coincides with a linear dichroism peak.⁹ The film was of high homogeneity and was hermetically sealed with a water lock to ensure high reproducibility.¹⁰ The concentration of 2,2'-bipyridyl could be decreased as desired by washing-out with chloroform.

An unstretched polyethylene film was inserted into the CD spectrometer (Jouan, Dichrographe Model B). In the light path behind this was placed the sample solution (0.01 cm *d*-fenchone). The film was stretched in steps until the apparent "CD" was zero. A quarter wave retardation had thus been obtained so that the

instrument was now adapted to measure the linear dichroism, $A_{\parallel} - A_{\perp}$. The sample was replaced by a film containing 2,2'-bipyridyl orientated with its plane perpendicular to that of the incident light. Its stretch direction relative to the stretch direction of the "quarter wave film" was then adjusted until the deflection recorded on the instrument was a maximum.

Table 1 shows a satisfactory agreement between corresponding linear dichroism values obtained from the two instruments, particularly after one set was adjusted by 0.006 absorbance units. This correction was presumably due to a systematic error in the absorbance measurement, since a plot of the CD against concentration yielded a line passing through the origin. This conclusion was also supported by the results of an experiment in which samples of different concentrations were preceded in the light path by a semi-oriented "quarter-wave" film. The apparent CD recorded with the elliptically polarized light was about half the real CD (without film) and was also linear with respect to concentration, the line again passing through the origin. The systematic error was thus not due to some deficiency in the photo-elastic quarter-wave plate. Furthermore the error cannot be due to stray-light from the Zeiss monochromator since this would produce the opposite effect.

A second series of experiments was conducted to test the instrument at very high dichroisms. Fig. 1 shows absorbance differences obtained with the technique described above, but at 500 nm with samples of acridine orange orientated in stretched polyvinyl alcohol (PVA).⁸ This

Table 1. Dichroisms, determined independently with two instruments, of a stretched polyethylene film containing various concentrations of orientated 2,2'-bipyridyl. For the absorption measurements, the estimated error in the last digit is given in parentheses. "Adjusted" refers to the correction necessary for the curve of $y=f(x)$ to pass through the origin.

Dichroism (Jouan)		Absorbance (Zeiss)		
$y = A_{\parallel} - A_{\perp} = LD$	A_{\parallel}	A_{\perp}	$x = A_{\parallel} - A_{\perp}$	adjusted
0.331	1.415	1.077	0.338(5)	0.332
0.140	0.705	0.559	0.146(3)	0.140
0.064	0.376	0.307	0.069(2)	0.063
0.031	0.230	0.192	0.038(2)	0.032
0.019	0.181	0.154	0.027(2)	0.021
0.017	0.171	0.147	0.024(2)	0.018

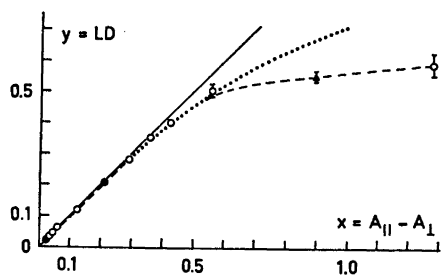


Fig. 1. Linear dichroism, y , obtained differentially (Jouan), plotted versus the difference, x , between separately determined $A_{||}$ and A_{\perp} (Zeiss), from stretched PVA/acridine orange films measured at 500 nm. ● two selected high-quality films suitable as standards, ▲ $A_{||} = 1.45$, $A_{\perp} = 0.51$, ... $y = 2(\log e) \tanh(x/2 \log e)$, — $x = y$. Deviation of ... from — at $x = 0.3$ is 4 %.

system is easier to handle than the first. In addition PVA retains its orientation and behaves as a clear, hard glass at room temperature, and much larger linear dichroisms can be produced. However, thickness and orientation cannot be reproduced as well as in the polyethylene/bipyridine system.

The CD spectrometer used here measures linear dichroisms lower than 0.3–0.4 with satisfactory accuracy but for larger absorbance differences it gives too low a value. Such behaviour could actually be expected because the instrument is designed to register a deflection proportional to the hyperbolic tangent of the dichroism divided by $2^{10} \log e$.⁵

For very high dichroisms the error exceeds even that expected on the above grounds. This is probably due to stray-light, depolarization, etc.

PVA-acridine orange films of high quality can be combined with quarter wave plates to provide permanent standards. The CD values of two such were found to be stable ($\pm 3\%$) for at least 5 months.

To eliminate effects due to different photomultiplier sensitivity when measuring the absorbance for the two orthogonal polarizations, a depolarizer may be inserted after the sample film. For a rapid test the sample film can be rotated 90° and the measurements repeated.

It is of interest to observe that the conventional standard with which the

CD spectrometer employed here was calibrated was quite satisfactory ("Isoandrosterone", Jouan-Quétin Co.).

The polyethylene matrix used was stretched approximately 300% and the PVA film 5–100%. The polyethylene quarter-wave films were more accurate at 280 nm than a tested commercial plate (Bernhard Halle Nachflg., Berlin, 300 nm). The quarter-wave films mounted as permanent standards were made of PVA stretched at about 80°C a little more than that required to give quarter-wave effect. Relaxation to give exact zero-CD deflection was attained by adjusting the temperature to about 70° in an air thermostat for intervals of a few minutes.

The second method employs as a standard a solid-state solution of *d*-10-camphor-sulphonic acid in PVA, which has been calibrated previously. It has proved to be very stable and is conveniently employed for regular checks of instrumental accuracy. Its use is certainly to be preferred to the "self-checking" systems of some instruments, e.g. the Jasco J-40 CD-spectrometer. Reproducibility of 0.5% is easily attained, and even under unfavourable conditions differences are always less than a few percent.

The standard is prepared as follows: 20 ml of 10% aqueous PVA solutions are mixed with 2 ml of 0.5 M aqueous *d*-10-camphorsulfonic acid. The resulting solution is centrifuged to remove bubbles and solid impurities from the PVA and then evaporated slowly (3 days) on a horizontal mirror glass, alternatively on a mercury surface. A piece is cut from the film for mounting either into a cell-holder or between quartz windows glued with a small amount of 10% PVA. The presence of strains can be detected with a polarizing microscope, but this is not necessary if the film is carefully handled. Typically, a 0.011 cm thick film yielding a CD peak at 295 nm of 0.0131 absorbance units is obtained. The absorbance is 0.8. A 0.5 M aqueous *d*-10-camphorsulfonate solution of the same thickness, gives a peak at 290 nm with CD 0.0121.¹ The difference is presumably due to solvent effects, PVA being similar to ethanol. The temperature dependence is less than $\pm 2\%$ for $\pm 5^\circ\text{C}$ at 24°C .

Certain CD instruments have recorders marked in ellipticity units, even though the instrument measures only the absorption difference $A_{||} - A_{\perp}$. It seems preferable, therefore, to recalibrate these in

terms of absorbance units using the relation $A_1 - A_r$ (CD in absorbance units) = θ (ellipticity in $^\circ$)/33.0.

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On the Structure of Gaseous Anisole

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Anisole is usually assumed to have a planar heavy atom skeleton,¹ but some authors have suggested that the carbon in the methyl group does not lie in the ring plane.²⁻⁵ Aroney *et al.*² measured the dipole moments and molar Kerr constants for anisole and some *para* substituted derivatives. They give an apparent torsional angle (ϕ) around the C₁-O₇ bond (see Fig. 1) of 18 $^\circ$ for anisole. An extended Hückel calculation⁴ gave minimum in energy for $\phi = 75^\circ$, while $\phi = 0^\circ$ was obtained by the CNDO/2 method if the methyl group was oriented to give minimum steric interac-

tion between the methyl hydrogens and the *ortho* hydrogens in the ring.⁵

Electron diffraction data of anisole were recorded for two nozzle temperatures, the low temperature data (about 55 $^\circ$ C) with Balzers Eldigraph KD-G2,^{6,7} and the high temperature data (about 250 $^\circ$ C) with the Oslo apparatus.⁸ In both cases two nozzle-to-plate distances were used, and composite intensity curves covering the *s*-ranges 2.0 - 28.5 Å^{-1} and 2.0 - 40.0 Å^{-1} were calculated in the usual way.⁹ The experimental radial distribution functions⁹ calculated by Fourier inversion of the intensity curves, are shown in Fig. 1.

The mean amplitudes of vibration (u) for both temperatures computed as described by Stølevik *et al.*,¹⁰ are included in Table 1. A simple force field, found to give u values for benzene in good agreement with more refined calculations,¹¹ was used for the phenyl group. The other force constants were also estimated from force constants found in related molecules.

Least-squares refinements of the structural parameters were then carried out with a diagonal weight matrix. Except for the CO bond the phenyl group was assumed to have hexagonal symmetry, and the methyl group to have a threefold symmetry axis coinciding with the CO bond. For the low temperature data good agreement between experimental and theoretical intensity values was obtained for a model with planar skeleton and mean amplitudes computed as described above. The angle α (Fig. 1) was first assumed to be zero, but better agreement was obtained for $\alpha = 4^\circ$. The most important u values were then refined as shown in Table 1. A slightly better fit was obtained. The radial distribution curve calculated with these parameters is given in Fig. 1A. A very slight improvement was obtained if ϕ was increased to about 10 $^\circ$.

For the high temperature data the fit was not satisfactory if ϕ was assumed to be zero and the mean amplitudes kept at the computed values (Fig. 1). Much better agreement was obtained if the u values were refined. However, the mean amplitudes for the distances from C₈ to the carbon atoms in the ring, became then considerably larger than the computed values.

Refinements were then carried out for various fixed values of ϕ ; the best agreement was obtained for $\phi = 40^\circ$. It seemed likely that the torsional oscillations about C₁-O₇ resulted in an apparent large devia-