who attempted to measure equilibrium distances of separation between surfaces immersed in ionic liquids.

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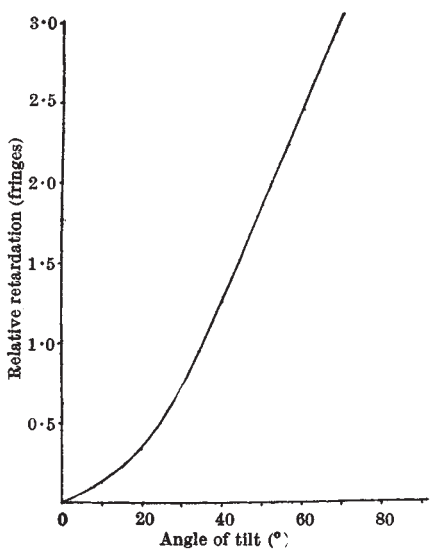
A Large Field Compensator of the Berek Type

P. E. Jellyman and A. J. Milne have described in *Nature* of March 27, p. 477, a form of photo-elastic compensator using a plate of *C.R.*39 resin in which a uniform stress variation (produced by pure bending) is frozen. A more sensitive compensator (which also depends on the frozen stress in *C.R.*39 resin) has been in use for more than a year at the Royal Aircraft Establishment. This instrument works on the same principle as the Berek compensator, in which a thin crystal slice (calcite or quartz), cut normal to the optic axis, can be tilted at various angles to the light path, giving a relative retardation increasing with the angle of tilt.

The Berek instrument is used in conjunction with a polarizing microscope. For covering fields of several inches diameter, natural crystals sufficiently large are not to be had. Instead, I have employed a plate of *C.R.*39, $\frac{3}{8}$ in. thick by about 4 in. by $2\frac{1}{2}$ in.

In the process of manufacture, the applied lateral pressure produces a frozen stress in a direction normal to the plate. This stress is not always uniform, but the piece selected had a variation of less than 3 per cent over its whole area. The *C.R.*39 sheet, which has uniform thickness, high polish and excellent light transmission, is lightly held between two frames mounted symmetrically on co-axial spindles which can rotate in a circular frame. The angle of rotation is shown by a pointer and scale. The whole frame is a sliding rotational fit in a split outer ring which is provided with an angular scale. Thus the axis of tilt can be adjusted to lie in the direction of either of the principal stresses. The inner ring is then fixed by the clamping screw. The instrument is set for compensation, and the plate is then tilted by means of a knurled knob; it can be clamped in any position. Owing to the large area of the plate it is rarely necessary to adjust the position of the instrument in the light field (except perhaps when the angle of tilt is high and the projected area is reduced).



Calibration curve for white light

One advantage of this type of instrument is that its sensitivity becomes greater for very small retardations, as is shown by the accompanying calibration curve. For a crystal slice, the slope of the curve would increase continuously. In the *C.R.*39 instrument, the slope appears to become constant. This may be due to the fact that the birefringence is not completely constant through the thickness of the material The instrument may be calibrated directly in Angström units if desired. The calibration has stayed constant for more than a year, and is not noticeably affected by slow temperature variations in the laboratory. It is necessary, of course, to avoid stresses due to clamping.

The range of the instrument is from 0 to 3 fringes (at about 69° angle of tilt), and its sensitivity 1/20 fringe or better. The calibration can be readily checked at any time.

When used, the instrument is mounted on the photo-elastic bench between the polarizer and the model, so as to avoid displacement of the image as a result of tilting.

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Isolation of a New Androstenol-3(α)-on-17 from the Urine of a Patient with Adrenal Cancer

In a previous paper (Dingemanse, Huis in 't Veld and De Laat[1]), we reported that we had detected, by means of a chromatographic-colorimetric method, a new 17-ketosteroid in the urine of patients suffering from a dysfunction of the adrenal cortex. This 17-ketosteroid, to which we refer in this paper as 17-ketosteroid II, occurs in the urine of normal individuals, as a rule, in small quantities only, and may be completely absent. Considerable amounts, however, have been found in the urine of persons with 'virilizing' tumours and with hyperplasias of the adrenal cortex.

This substance has now been isolated from the urine, and obtained in pure condition. We used for this purpose 2 litres of urine of a female child aged two, who showed symptoms of virilism, and from whom afterwards an adrenal tumour was extirpated.

The urine was neutralized and extracted according to the method of Dingemanse and Laqueur[2], and the extract was purified by means of chromatographic analysis and high-vacuum sublimation. After re-

peated crystallization from a watery methanol solution and from petroleum ether, a product with a melting point of 140·5–141° C. was obtained. In ethanol its specific rotation was found to be $+121 \pm 3°$.

The quantitative analysis led to the empirical formula $C_{19}H_{28}O_2$ (H, 9·68; C, 79·06 per cent). In 80 per cent ethanol it gave no precipitate with digitonin. Titration, by the method of Rosenmund – Kuhnhenn, revealed the presence of one double bond (1·89 and 2·14 equivalents of bromine were taken up). With hydroxylamine it was found to form a monoxime with a melting point of 109–113° C. (N, 4·40 per cent). An acetate could not be isolated in the pure condition. The α-ketosteroids are, as is well known, more difficult to esterize than the β-ketosteroids.

The new ketosteroid proved to be unstable in the presence of mineral acids. When treated at room temperature with hydrochloric acid, it formed two transformation products that could be separated by chromatographic analysis. The first proved to have a melting point of 151–152° C., and the quantitative analysis led to the empirical formula $C_{19}H_{27}OCl$ (C, 74·44; H, 8·83; Cl, 11·53 per cent).

The second transformation product had a melting point of 140–141° C., and gave a precipitate with digitonin. When this substance was mixed with dehydro-*iso*-androsterone, the melting point underwent no change. Its acetate proved to have a melting point of 171–172° C.; mixing with dehydro-*iso*-androsterone acetate did not lower the melting point. We must assume, therefore, that it is identical with Δ5-androstenol-3(β)-on-17 (that is, dehydro-*iso*-androsterone).

Our idea of the reaction with hydrochloric acid is that it takes place as follows:

17-ketosteroid II $\xrightarrow{HCl}$ chloro-ketone $\xrightarrow{HCl}$ dehydro-*iso*-androsterone.

Dehydro-*iso*-androsterone is *not* appreciably transformed in a chloroketone when it is treated at room temperature with hydrochloric acid. Butenandt[3,4] has already pointed out in a paper on the chloroketone of male urine that the precursor of this compound may be different from dehydro-*iso*-androsterone.

The configuration of 17-ketosteroid II must therefore be very similar to that of dehydro-*iso*-androsterone. For this reason it seems to us that the atom C_3 will bear an α-hydroxyl group. For a keto group at C_{17} the Zimmermann reaction is characteristic. The Pincus reaction of 17-ketosteroid II is qualitatively as well as quantitatively the same as that of dehydro-*iso*-androsterone.

On account of the fact that 17-ketosteroid II is transformed by the action of hydrochloric acid in dehydro-*iso*-androsterone, there are apparently three positions in which the double-bond might, theoretically, be present; namely, Δ4.5, Δ5.6 and Δ6.7. Ruzicka and Goldberg[5] have synthesized the Δ5.6-androstenol-3(α)-on-17, which proved to have a melting point of 221° C., and $[\alpha]_D \sim 0°$. Our new ketosteroid, therefore, cannot be identical with this substance.

As the formula Δ4.5-androstenol-3(α)-on-17 accounts better than the other ones for the instability of our substance in the presence of acids (Wolfe, Fieser and Friedgood[6]), this configuration seems to be the most plausible. Wolfe *et al.* have already pointed out that this ketosteroid might be found in urine extracts.

We have tried to ascertain the configuration of 17-ketosteroid II by oxidation, using Oppenauer's method, of the hydroxyl group attached to C_3. If the formula suggested above be correct, this ought to give Δ4.5-androstenedione. The melting point of our oxidation product proved, however, to be 185° C., and is therefore different from the melting points that have been reported for Δ4.5-androstenedione. Mixed with Δ4.5-androstenedione it showed, moreover, a sharp fall in its melting point.

The possibility that our 17-ketosteroid II might be an *i*-steroid was also considered. *I*-steroids are so far known only as synthetic products (Wallis, Fernholz and Gephart[7]). When a surplus of bromine is added, such substances ought to give tribromides (Beyon, Heilbron and Spring[8]; Wallis *et al.*[7]), whereas our product absorbed in the Rosenmund – Kuhnhenn titration but two equivalents of bromine

The investigation is being continued.

The quantitative analyses were carried out by Mr. P. Hubers in the Laboratory for Organic Chemistry of the Amsterdam Municipal University. A grant was received for this study from Organon, Ltd., Oss.

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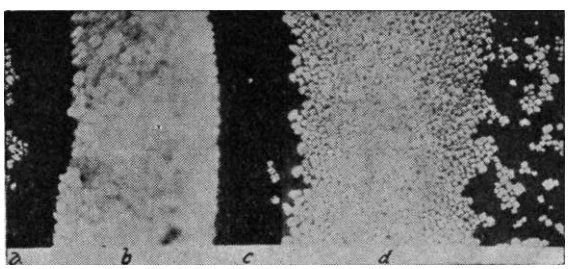
Evidence for an Electrostatic Field on Penicillin Assay Plates

ONE of the effects of penicillin on susceptible bacteria is to cause a change in electrostatic charge which may be measured in terms of ζ potentials, and which it has been suggested may be used quantitatively for assaying solutions of penicillin[1,2,3]. So early as 1938, Loiseleur[4] studied changes that occur in electrostatic charge during proteolysis, such as would be expected during lysis of bacterial cells. Similar changes in charge resulting in development of an electrostatic field can be shown on penicillin assay plates by treatment with electronegative or electropositive colloids suspended in buffers at *p*H levels above or below the isoelectric range of the nucleoproteins.

The accompanying reproduction is of a photomicrograph of a segment of an inhibition zone on a standard assay plate that was flooded for a few minutes with a suspension of starch in phosphate



SEGMENT OF AN INHIBITION ZONE SHOWING FLOCCULATION OF STARCH WITHIN ZONE