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Separation and determination of Z and E isomers of dodecyl urocanate

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(E)-Urocanic acid, a major metabolite of histidine [1,2] and a naturally occurring sunscreen, accumulates in the skin and is eventually excreted in sweat [3,4]. This compound, which undergoes $E \rightleftharpoons Z$ photoisomerization, is one of the major UV light absorbers in the epidermis [5,6]. Recent studies, however, have revealed additional photobiological properties, i.e., photochemical binding to DNA and photoimmunosuppressive activity for the Z isomer [7-10]. These observations rule against the use of urocanic acid as a sunscreen and led us to undertake a study of urocanic acid long-chain esters.

![Chemical structures of E and Z isomers of urocanic esters](image)

The separation of the E and Z isomers of urocanic esters has a twofold interest: photochemical, as it is necessary for the study of $E \rightleftharpoons Z$ isomerization and of the possible photocycloadditions; and biological, for the study of the influence of the configuration on the immunosuppressive properties.

Although separations of urocanic acid [11-14] and methyl urocanate isomers have been reported [15], those of for long-chain esters have not. We therefore set out to separate the E and Z isomers of $n$-dodecyl urocanate and to determine the proportions of mixtures of these compounds by chromatography.
EXPERIMENTAL

Instrumentation and procedure

Photoisomerization reactions were carried out in a Rayonet-type reactor (New England Ultraviolet) at 254 nm using 10-ml quartz tubes on a rotating rack.

Thin-layer chromatography (TLC) was done on 2.5 × 7.5 cm silica gel 60 Å plates (250-µm layer) (Whatman) with UV detection at 254 nm. The solvent was chloroform–methanol (95:5, v/v).

A 20 × 2 cm I.D. column of silica gel (250–400 mesh) (Fluka) was used for the preparative separation of the Z isomer from a 160-mg mixture of Z and E isomers (Z:E ≈ 75:25) using chloroform–methanol (95:5, v/v) as eluent. The elution was followed by TLC. The first isomer appeared after 75 ml of eluent and was pure in the twelve following 3.5 ml-fractions collected.

High-performance liquid chromatography (HPLC) was performed using a system consisting of a Millipore Waters unit (Model 510) with an automatic gradient controller, a Waters Assoc. 990 photodiode array detector and a Waters Assoc. μPorousil (10 µm) column (30 cm × 4 mm I.D.). The detection wavelength was set at 310 nm, where both isomers absorb. The eluent was chloroform–ethanol (HPLC grade) (85:15, v/v) after having been filtered over Millipore membranes (0.22 µm). The flow rate was 0.7 ml/min.

UV spectra were recorded on a Hewlett-Packard HP 8451 A spectrophotometer. IR spectra were recorded on an FT-IR Perkin-Elmer 1760 X spectrophotometer. NMR spectra were recorded in deuterochloroform on a Bruker AC 80 apparatus.

Chemicals

(E)-Dodecyl urocanate was obtained by esterification [16] of urocanic acid (Aldrich). Its purity was checked by TLC ($R_F = 0.21$).

Photoisomerization was achieved by a 2-h irradiation, at 254 nm of a 6.5 · 10⁻³ mol l⁻¹ solution of 160 mg of (E)-dodecyl urocanate in 80 ml of butanol at 35°C.

After having evaporated the solvent, the separation of the Z isomer was performed by column chromatography using the procedure described before. The twelve fractions containing the isomer eluted first were evaporated to give 110 mg of a solid of m.p. 61°C (uncorrected). This compound obtained in ca. 90% yield, was identified as the Z isomer by TLC ($R_F (E) = 0.21; R_F (Z) = 0.44$) and ¹H NMR spectroscopy:

\[
\text{–CH} = \text{CH–COO(CH}_2)_1\text{CH}_3: \\
\text{E isomer, } \delta H_\alpha = 6.73 \text{ ppm, } \delta H_\beta = 7.58 \text{ ppm, } \text{J(H}_\alpha, H_\beta) = 16 \text{ Hz; Z isomer, } \delta H_\alpha = 5.60 \text{ ppm, } \delta H_\beta = 6.83 \text{ ppm, } \text{J(H}_\alpha, H_\beta) = 13 \text{ Hz.}
\]

The UV spectra were recorded in butanol: E isomer, $\lambda_{\text{max}} = 292 \text{ nm, } \epsilon = 19 \text{ 200 l mol}^{-1} \text{ cm}^{-1};$ Z isomer, $\lambda_{\text{max}} = 302 \text{ nm, } \epsilon = 17 \text{ 100 l mol}^{-1} \text{ cm}^{-1}.$

The IR spectra were recorded in carbon tetrachloride: E isomer, $v_{\text{NH}}(\text{free}) = 3470 \text{ cm}^{-1} \text{ (sharp band), } v_{\text{NH}}(\text{bonded}) = 3200–3425 \text{ cm}^{-1} \text{ (intramolecular); Z isomer, } v_{\text{NH}}(\text{bonded}) = 3258 \text{ cm}^{-1} \text{ (intramolecular hydrogen bond).}

RESULTS AND DISCUSSION

The HPLC separation of an artificial mixture of (Z)- and (E)-dodecyl urocanate is shown in Fig. 1. Under the conditions described above, the elution times were 15.25 min and 6.75 min for the Z and E isomers, respectively. The weaker retention of the Z
Fig. 1. HPLC separation of an artificial mixture of (Z)- and (E)-dodecyl urocanate isomers. Column, µPorasil (10 µm) (30 cm × 4 mm I.D.); detection, absorption at 310 nm; mobile phase, chloroform-ethanol (85:15); flow-rate, 0.7 ml/min; temperature = 20°C; injection: 15 µl of an approximately 10⁻³ mol l⁻¹ solution. Values on the y-axis are absorbance units.

isomer is probably related to the strong intramolecular hydrogen bond in this molecule, which is readily seen in infrared spectroscopy. This bond, already reported for methyl urocanate [17], is not disrupted in the long-chain ester.

![Urocanate structure](image)

The peaks obtained in HPLC were assigned by analysing the previously identified pure isomers separately. Quantitative HPLC was then undertaken. Calibration was performed by plotting known concentrations of each isomer against peak area. Unlike urocanic acid [18], the isomers did not show a deviation from the Beer-Lambert law in the concentration range tested (seven concentrations from 4 · 10⁻⁵ to
This HPLC method was tested using two artificial mixtures of \( Z \) and \( E \) isomers, whose proportions were measured by weighing and chosen in the range expected in photochemical experiments (45% \( \leq \) \( Z \) \( \leq \) 75%). The results obtained, given in Table I, illustrate the precision of the method.

### Table I

<table>
<thead>
<tr>
<th>Mixture</th>
<th>( Z:E ) (weighing)</th>
<th>( Z:E ) (HPLC)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1 ) ( (Z:E \approx 3) )</td>
<td>3.33 ± 0.03</td>
<td>3.35 ± 0.02</td>
</tr>
<tr>
<td>( 2 ) ( (Z:E \approx 1) )</td>
<td>0.92 ± 0.01</td>
<td>0.91 ± 0.01</td>
</tr>
</tbody>
</table>

* Values ± S.D. \((n = 4)\).

Having confirmed the precision of the HPLC analysis for these compounds, we set out to analyse the mixture obtained after a 2-h irradiation of a \( 6.5 \times 10^{-3} \) \( \text{mol l}^{-1} \) solution of \((e)\)-dodecyl urocanate in butanol. Eight tubes were used in the rotating rack and analysed separately (two measurements for each tube) by HPLC and \(^1\)H NMR spectroscopy. The following results were obtained: \( Z:E \) (HPLC) between 2.58 (72.1:27.9) and 2.62 (72.4:27.6) and \( Z:E \) (\(^1\)H NMR) between 2.57 (72:28) and 3.00 (75:25). The results obtained by the two methods are close, but those given by HPLC are more reproducible.

We therefore decided to use HPLC to determine the photostationary state of the photoisomerization of \((E)\)-dodecyl urocanate in butanol \((6.5 \times 10^{-3} \text{mol l}^{-1})\), and found that it was reached after 1 h of irradiation and corresponded to the ratio \( Z:E = 2.6 \pm 0.02 \).

### CONCLUSION

The preparative method for the separation of \((Z)\)- and \((E)\)-dodecyl urocanate described has proved to be a readily applicable technique that allows the rapid production of large amounts of these two isomers. This will enable us to continue the study of the biological activities of these compounds, for instance the immunosuppression phenomenon. The quantitative analysis by HPLC of \( E \) and \( Z \) isomer mixtures greatly facilitates the study of the photoisomerization of dodecyl urocanate in a wide range of solvents and concentrations.

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### REFERENCES