Table II—Response Factors of I–IV with Respect to the Internal Standard [a]

Solution, %	Component	Response Factor	*RSD*, %
80	I	2.06	0.78
	II	1.79	0.71
	III	1.40	1.11
	IV	0.99	0.64
100	I	2.05	1.02
	II	1.81	0.82
	III	1.41	1.00
	IV	0.99	1.74
120	I	2.10	0.90
	II	1.84	1.14
	III	1.43	1.68
	IV	1.04	1.43

[a] Three solutions were prepared, and 18 measurements were made.

Table III—Precision Study of a Commercial Preparation

Parameter	Compound I	II	III	IV
Theoretical content, mg/g	13.3	13.3	12.5	18.3
$\bar{X}$ (n = 8)	13.0	13.1	12.3	18.3
RSD, %	0.75	0.76	1.2	1.2

RESULTS AND DISCUSSION

The recovery study was conducted by preparing a standard solution containing the internal standard and all of the ingredients in amounts as found in the commercial product. The solution was determined 10 times. The results (Table I) show that active ingredients can be separated (Fig. 1) and analyzed by direct injection into the chromatograph. Eventual interferences from other ingredients were investigated by preparing a solution that excluded the active components. The chromatogram of this solution showed a peak of one component[7] with a retention time at ~9.15 min, which did not interfere. Peaks of less volatile compounds[8] appeared between 25 and 28 min. Therefore, it was convenient to allow the instrument to run periodically for 30 min during the analysis.

The ratios of the area per weight of substance to the area per weight of internal standard were calculated for the active components over a range of 80–120% of the label claim. Results for three solutions (80, 100, and 120%) are reported in Table II. Several liquid phases were tried to accommodate compounds of different polarity in the same chromatogram. The response factors throughout the concentration range studied attest to the usefulness of OV-225 as the liquid phase.

Since the procedure was planned only to determine the active ingredients contained in the alcoholic base, it appeared preferable to remove first the propellent from the rest of the sample at a temperature (~0°) where the alcoholic vapor tension was considerably reduced. The residual propellent was eliminated by shaking the decanted sample for 20 min. Precision also was examined by analyzing a production lot commercial preparation. Results are reproduced in Table III.

The method presented is reasonably fast, and precision is consistent with accuracy.

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[7] Acetylated lanolin alcohol (Crodalan).

[8] Polyol fatty acid esters (Cetiol HE) and triglyceride mixture of saturated fatty acids (Myglyol 812).

Optical Characterization of a Low Solubility Organic Compound

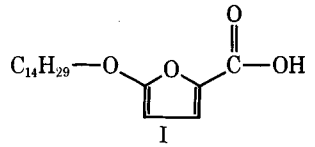
H. E. HUBER

Received April 7, 1980, from *Merrell Research Center, Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, OH 45215.* Accepted for publication March 5, 1981.

Abstract □ The X, Y, and Z principal vibration directions along with the principal refractive indexes, optic angle, optical sign, birefringence, optical orientation, and crystal system for the low solubility compound 5-(tetradecyloxy)-2-furancarboxylic acid were determined with a polarizing microscope and spindle stage. The X and Z principal vibration directions are not coincident with the a and c crystallographic axes; however, the Y direction is considered to be coincident with the b axis. Therefore, the crystal is assigned to the monoclinic crystal system. The bladed/lath-shaped crystals rest on one of the two large orthopinacoid (100) faces and present the microscopist with a single plane of optical symmetry. A β refractive index of 1.555 is observed with the crystal axis of elongation parallel to the polarizer, and a γ' of ~1.600–1.660 is observed in the contiguous extinction position. Determination of the optic angle, principal vibration directions, and principal refractive indexes was facilitated by mounting the crystals on a spindle stage for rotation about the b crystallographic axis (optic normal).

Keyphrases □ 5-(Tetradecyloxy)-2-furancarboxylic acid—optical characterization using a polarizing microscope and spindle stage □ Optical characterization—low solubility compound, 5-(tetradecyloxy)-2-furancarboxylic acid □ Crystallography—optical characterization of a low solubility organic compound, 5-(tetradecyloxy)-2-furancarboxylic acid

Control of dosage formulation in the clinic by conventional aqueous dissolution methods was not possible with 5-(tetradecyloxy)-2-furancarboxylic acid[1] (I) because of its poor solubility in alcohol and/or water. A high concentration of surfactant and water was eventually utilized for the solvent, but other controls were necessary to ensure that changes in the crystalline structure between lots did not occur. Long-chain fatty acid compounds such as this one are well known for their polymorphic character (1).



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[1] RMI 14,514, Merrell Research Center.

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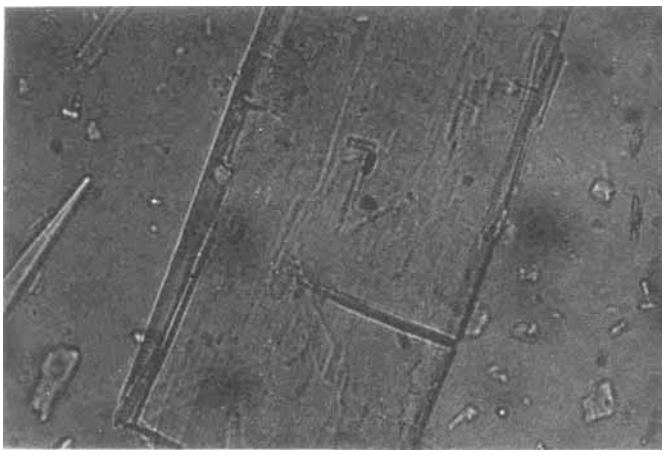

Figure 1—*Photomicrograph of I crystals obtained with the tungsten filament lamp at 4 amp and 0.25-sec exposure time. No filters were used. Crystals were mounted in 1.460 Cargille fluid, A 1/5 series.*

BACKGROUND

Some investigators (2–4) attempted to control this variable with differential scanning calorimetry or related thermal methods, and others utilized X-ray diffraction. In a few isolated reports (5–7), a combination of these methods, along with optical crystallographic characterizations, was used.

Once the crystal is characterized, optical methods offer many advantages for routine identification of other lots. Optical values are quite specific for a substance, and most microscopic examinations can be performed with a minimum of time and effort. The number of discriminating optical parameters that can be used is usually numerous; consequently, one or more can be selected as conditions or limitations dictate.

An optical characterization of I is presented here and was utilized along with surface area measurements to control potential crystal changes. An X-ray diffraction profile was determined as part of the total crystal characterization; however, only optical methods are utilized for routine examination.

Geologists and chemists have long relied on optical methods for ready identification and, in some cases, composition determinations of unknown mineral or chemical samples. Each substance has a unique atomic arrangement and, therefore, unique optical properties. Optical determinative data for organic compounds are limited compared to those for inorganic materials; however, the data available indicate that the same specificity applies.

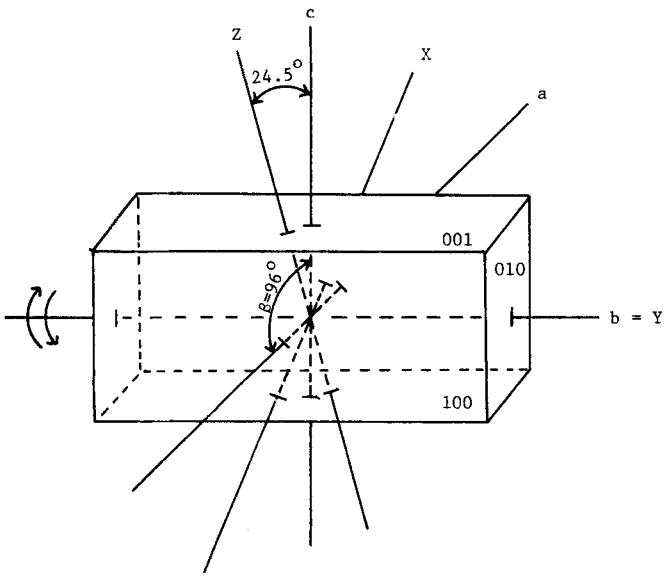

Figure 2—*Optical orientation of I in relation to its crystallographic axes.*

Table I—Optical Properties Obtained for I

Parameter	Value
Refractive indexes (5893A – D line 25°)	
α	1.470 ± 0.002
β	1.555 ± 0.002
γ	1.670 ± 0.002
Birefringence	0.200
Optic axial angle (5893A – D line 25°)	$2V = 81° \pm 2.0$
Optical axial plane	010
Sign of double refraction	Positive
Extinction	Parallel
Crystal system	Monoclinic
Elongation sign	±

Microscopic slide mounts of unmilled I exhibited 100–500-μm lath-shaped crystals (Fig. 1) that appeared suitable for the study. By utilizing polarizing microscopic procedures, optical crystallographic characterization of I was successfully performed.

EXPERIMENTAL

Compound I was obtained by crystallization from methyl ethyl ketone. One lot of crystals was used for the crystal characterization study, and other lots were examined to verify that the optical data were consistent.

Equipment—A trinocular widefield microscope[2] was fitted with a polarizing substage condenser, an analyzer in the body tube, strain free 0.40 and 0.65 N.A. objectives, and a 12-v, 60-w tungsten filament lamp. A regulating transformer permitted the load to be varied up to a maximum of 6 amp. Photomicrographs were obtained with a 35-mm camera[2] mounted on the vertical ocular. Film[3] was 35-mm black and white with an ASA of 125. A second monocular, polarizing microscope[2] with an adjustable Bertrand lens and iris in the body tube, strain-free objectives up to 0.85 N.A., and a tungsten filament lamp with heat filters also was used.

The stage was a simple, inexpensive device described previously (8) in which a crystal is attached to a spindle and rotated about a single axis perpendicular to the microscope axis.

Microscopic Examination—An amount of powder adhering to the point of a dissecting needle was tapped onto the surface of a microscope slide, and the index of refraction fluid was applied with a glass applicator. The powder was mixed with fluid, and a cover slip was added. The slide was then examined with a polarized light microscope using orthoscopic and conoscopic optic conditions. (For an extensive and detailed description of microscopic procedures for optical crystallographic studies, see Refs. 9–11 or any of the numerous publications available on the

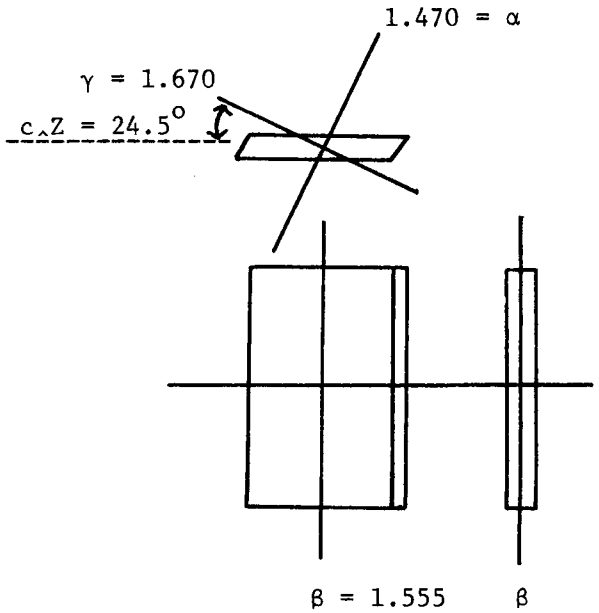

Figure 3—*Orthographic projection of typical crystal of I.*

[2] Leitz.
[3] Kodak PX135.

subject.) Spindle stage examination of crystals followed that described previously (8, 11).

RESULTS AND DISCUSSION

Preliminary orthoscopic examination revealed that the lath-shaped crystals (Fig. 1) exhibited a single plane of optical symmetry from which a single β refractive index value could be obtained. The crystals were quite thin, and most grains lay on the 100 orthopinacoid.

To obtain β and γ refractive index values, the crystals were mounted (Fig. 2) onto a spindle stage. The crystals were rotated on their b-axis to orientations that presented the principal vibration directions, and refractive index values were determined by immersion in various fluids.

Crystals could not be oriented to provide a 010 end view, and therefore, a β-angle could not be observed or measured microscopically. Subsequent data reported from X-ray diffraction methods gave a value of 96°. An orthographic projection of a typical crystal of I is shown in Fig. 3, and Table I presents the optical properties.

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Synthesis of Dehydroepiandrosterone Sulfatide and 16α-Halogenated Steroids

MAGID ABOU-GHARBIA, LAURA PASHKO, ARTHUR SCHWARTZ, and DANIEL SWERN ˣ

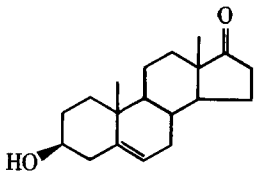
Received December 8, 1980, from the *Fels Research Institute and Department of Chemistry, Temple University, Philadelphia, PA 19122.* Accepted for publication March 5, 1981.

Abstract □ Dehydroepiandrosterone sulfatide was prepared in a 68% yield by the reaction of 5-androstene-3β-ol-17-one 3-sulfate (silver salt) with dipalmitoyl α-iodopropylene glycol. The sulfatide was found to be a more potent inhibitor of human glucose-6-phosphate dehydrogenase than dehydroepiandrosterone. 16α-Halogenated steroids also were prepared by direct halogenation of the steroid or indirect halogenation of an appropriate steroidal intermediate. Among various halogenated steroids, 16α-bromoepiandrosterone was ~50 times as potent as dehydroepiandrosterone as an inhibitor of glucose-6-phosphate dehydrogenase.

Keyphrases □ Dehydroepiandrosterone sulfatide—synthesis, tested as glucose-6-phosphate dehydrogenase inhibitor □ 16α-Halogenated derivatives of epiandrosterone—synthesis, tested as glucose-6-phosphate dehydrogenase inhibitors □ Glucose-6-phosphate dehydrogenase—synthesis of dehydroepiandrosterone sulfatide and 16α-halogenated steroids

Of the naturally occurring steroids that had been tested, dehydroepiandrosterone (I) proved to be an outstanding noncompetitive inhibitor of glucose-6-phosphate dehydrogenase (1, 2). The sulfated form of I (Ia) is a major adrenal secretory product in humans. Approximately 99% of the plasma form of this steroid is sulfated, while the remainder is unconjugated steroid. The plasma concentration of the sulfated form of I exceeds that of any other steroidal hormone, yet its biological role is unknown. (Most investigators refer to the conjugated form as the sulfate.)

According to Oertel and coworkers (3, 4), the sulfated form of the hormone found in human plasma is I-sulfatide (II), the ester of sulfatidic acid, which must be isolated under extremely mild conditions because of its lability. In



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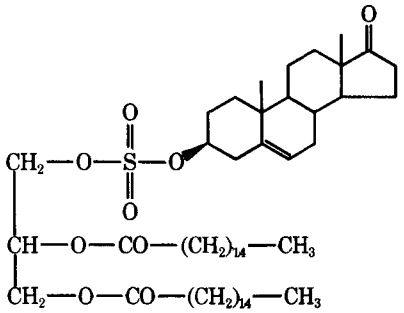

II

the search for analogs of considerably higher potency than I or II in inhibiting glucose-6-phosphate dehydrogenase to diminish or eliminate undesirable side effects of high dosage dehydroepiandrosterone therapy, the sulfatide (II) and 16α-halogenated derivatives of epiandrosterone were synthesized.

RESULTS AND DISCUSSION

Dipalmitoyl-L-α-iodopropylene glycol (III) was prepared in an 80% yield by facile acylation of L-α-iodopropylene glycol with two equivalents

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