Convenient Synthesis of Alkyl Esters of Urocanic Acid

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Abstract □ Ethyl, *n*-dodecyl, and *n*-hexadecyl esters of urocanic acid (4-imidazoleacrylic acid) were prepared from 4-imidazolecarboxaldehyde in satisfactory yields *via* the Wittig reaction.

Keyphrases □ Urocanic acid—alkyl esters, synthesis *via* Wittig reaction □ Wittig reaction—synthesis of alkyl esters of urocanic acid □ Sunscreens, potential—urocanic acid, synthesis of alkyl esters by Wittig reaction

The finding that urocanic acid (4-imidazoleacrylic acid), a metabolite of histidine, occurs in human epidermis (1) has generated interest in the possible use of the acid and its alkyl esters in cosmetics as sunscreens (2–4). *trans*-Urocanic acid, which readily isomerizes to *cis*-urocanic acid when irradiated with UV rays (5), was reported to show excellent sunlight-screening action since it has noteworthy absorption in the UV range of 240–320 nm, which is similar to the erythemal action spectrum (6). However, the utilization of urocanic acid is restricted because it is almost insoluble in oils or water unless it is derivatized to a proper salt, which, in turn, is readily removed by washing or perspiration. In contrast, most urocanic acid esters are soluble in most oils, which adds to their practical applicability.

Alkyl esters of urocanic acid generally have been synthesized by condensation of 4-imidazolecarboxaldehyde with malonic acid to give urocanic acid, which subsequently is esterified (2, 7–10). However, this two-step sequence is time consuming and deficient in overall yield. Although urocanic acid also is produced by enzymatic deamination of L-histidine (11), esterification of the acid with long-chain aliphatic alcohols is difficult due to the poor solubility of the acid in alcohols and organic solvents.

The present report describes a convenient one-step synthesis of some alkyl esters of urocanic acid from 4-imidazolecarboxaldehyde *via* the Wittig reaction.

EXPERIMENTAL

Spectral Measurement—IR spectra[1] were obtained on potassium bromide disks. The PMR spectrum was recorded on a 100-MHz spectrometer[2] with tetramethylsilane as the internal standard. The chemical shifts and coupling constants are represented by δ and Hertz units, respectively. Mass spectra[3] were measured at an ionizing potential of 25 ev.

Chromatography—The high-performance liquid chromatograph[4] was equipped with a UV detector and a 500 × 3-mm i.d. glass column packed with vinyl acetate porous polymer gel[5]. The analyses were carried out with 0.75% (w/w) methanolic triethylamine as the eluent and naphthalene as the internal standard. The column eluates were monitored at 288 nm.

Materials—4-Imidazolecarboxaldehyde (I), prepared according to the procedure reported by Pyman (12), was obtained as pale-yellow crystals, mp 173–174° [lit. (13) mp 173–174°]. Diethyl carbethoxymethylphosphonate[6] (II) was obtained commercially and used without further purification. Carbethoxymethylenetriphenylphosphorane (III), mp 118–119° [lit. (14) mp 116–117°], and carbo-*n*-hexadecyloxymethylenetriphenylphosphorane (IV), mp 90–91.5° [lit. (14) mp 90–91°], were prepared by the method of Isler *et al.* (14). Carbo-*n*-dodecyloxymethylenetriphenylphosphorane (V) was prepared in an analogous manner, yielding white crystals, mp 44.5–45.5°. All melting points are uncorrected.

Anal.—Calc. for $C_{32}H_{41}O_2P$: C, 78.66; H, 8.46; P, 6.34. Found: C, 78.37; H, 8.47; P, 6.29.

Preparation of Ethyl Urocanate (VI)—*Method A*—To a stirred solution of the aldehyde (I) (1.92 g, 20 mmoles) and the phosphonate (II) (6.73 g, 30 mmoles) dissolved in 20 ml of *N,N*-dimethylformamide was added 0.96 g (40 mmoles) of sodium hydride at room temperature. After gas evolution had ceased, the mixture was heated to 95° and held at that temperature for 3 hr. After removal of the solvent at reduced pressure, the residue was acidified with 5% HCl with stirring and extracted with benzene (30 ml) to eliminate excess phosphonate.

The aqueous layer was made basic with dilute sodium bicarbonate solution and extracted with two 15-ml portions of chloroform. The combined chloroform extracts were washed with water, dried over calcium sulfate, and concentrated to afford a pale-brown oil. After dehydration[7] at 95° for 2 hr *in vacuo* and recrystallization from chloroform–petroleum ether, this oil gave 1.43 g of VI (43% yield) as white crystals, mp 81–82°; IR: 1705 (C=O) cm^{-1}; PMR ($CDCl_3$): δ 1.29 (t, 3H), 4.22 (q, 2H), 6.41 (d, 1H, J = 16 Hz), 7.32 (s, 1H), 7.60 (d, 1H, J = 16 Hz), and 7.74 (s, 1H).

Anal.—Calc. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 57.73; H, 6.06; N, 16.89.

Method B—A mixture of the aldehyde (I) (1.92 g, 20 mmoles), the phosphorane (III) (8.36 g, 24 mmoles), and dimethyl sulfoxide (25 ml) was maintained at 130° for 3 hr. Evacuation of dimethyl sulfoxide *in vacuo* and similar workup gave 1.73 g (52% yield) of VI.

Preparation of *n*-Dodecyl Urocanate (VII)—A mixture of the aldehyde (I) (1.92 g, 20 mmoles), the phosphorane (V) (11.73 g, 24 mmoles), and dimethyl sulfoxide (25 ml) was heated at 140° for 4 hr. To the reaction mixture was added 10% HCl (10 ml), and the mother liquor was evaporated *in vacuo* to dryness. The residual paste was washed with acetone–isobutanol, suspended in hot water (10 ml), and treated with dilute sodium bicarbonate solution to give a brown oil, which gradually crystallized on standing.

The crystals were collected, washed with water, dehydrated at 80° for 2 hr *in vacuo*, and recrystallized from petroleum ether to yield 3.55 g of VII (58% yield) as white crystals, mp 61–62°; IR: 1705 (C=O) cm^{-1}; mass spectrum: *m/e* 306 (M^+, 44%), 277 (11), 179 (30), 138 (75), 122 (50), 121 (71), 95 (58), 94 (100), and 82 (59).

Anal.—Calc. for $C_{18}H_{30}N_2O_2$: C, 70.55; H, 9.87; N, 9.14. Found: C, 70.27; H, 9.90; N, 9.14.

In a similar manner, *n*-hexadecyl urocanate[8] (VIII) was obtained as pale-yellow crystals (54% yield), mp 55–56°; IR: 1705 (C=O) cm^{-1}; mass spectrum: *m/e* 362 (M^+, 59%), 333 (20), 179 (40), 138 (53), 122 (69), 121 (64), 95 (65), 94 (63), and 82 (100). The mass calculated for $C_{22}H_{38}N_2O_2$ was 362.2933; the mass found was 362.2945.

RESULTS AND DISCUSSION

Although there have been several reports of the reaction of imidazolecarboxaldehydes with nucleophilic reagents (12, 15, 16), the Wittig reaction of the aldehydes generally has not been used. The reaction of 8-xanthinecarboxaldehyde, which contains the 2-imidazolecarboxal-

[1] Hitachi 215 spectrometer.
[2] Japan Electron Optics Laboratory model JNM-MH 100 instrument.
[3] Japan Electron Optics Laboratory model JMS-D 300 mass spectrometer.
[4] Hitachi 635.
[5] TSK GEL LS-140, Toyosoda Co.
[6] Aldrich Chemical Co.
[7] Ethyl urocanate is known to form a hemihydrate when isolated from aqueous media. In the present study, the crude product was dehydrated to obtain the anhydrous ester; otherwise, a mixture of the two usually was obtained. Dehydration proceeded smoothly by heating the hydrated ester at fusion temperature *in vacuo*.
[8] Gas chromatographic and mass spectrometric analyses showed that the product contained ~5% of the *n*-tetradecyl ester and 10% of the *n*-octadecyl ester.

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dehyde moiety, with carbethoxymethylenetriphenylphosphorane was reported (17). Thus, the aldehyde (I) was subjected to the reaction with the phosphonate (II) or the phosphorane (III) to yield the ethyl ester (VI) in satisfactory yield. In the reaction of I with II (Method A), 2 equivalents of sodium hydride were used, and aprotic polar solvents such as dimethyl sulfoxide and *N,N*-dimethylformamide were necessary to dissolve the sodium salt of I. The time conversion was monitored by high-performance liquid chromatography (HPLC), and I was converted to VI in a 58% yield when the reaction was carried out with 1.5 equivalents of II at 95° for 3 hr in *N,N*-dimethylformamide. The reaction rate was slower in dimethyl sulfoxide.

On the other hand, I reacted readily with resonance-stabilized ylide III (Method B) in various solvents capable of dissolving I to yield VI. However, the reaction in ethanol resulted in a lower yield than in aprotic solvents, probably due to decomposition of III by a side reaction with ethanol. Of the several solvents used, dimethyl sulfoxide and diglyme were the most suitable for this reaction. The yields of VI for the reaction in dimethyl sulfoxide and in diglyme were 66 and 63%, respectively (molar ratio of III to I of 1.2, 130°, 3 hr).

From the viewpoints of utility and facility, these results indicate that carboalkoxymethylenetriphenylphosphoranes are more appropriate for the preparation of esters of imidazoleacrylic acids containing an acidic imino hydrogen atom. Method B thus was applied to the preparation of long-chain alkyl esters. The aldehyde (I) was allowed to react with the phosphorane (V), and HPLC analysis of the reaction mixture showed the formation of *n*-dodecyl ester (VII) in a 70% yield. Similarly, I was converted, by the reaction with the phosphorane (IV), to *n*-hexadecyl ester (VIII) in 66% yield.

These esters (VI–VIII) were soluble in most oils and had an absorption maximum at 290 nm in 0.75% (w/w) triethylamine in methanol.

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Simultaneous Determination of Niacinamide, Pyridoxine, Riboflavin, and Thiamine in Multivitamin Products by High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic assay was developed for the determination of four water-soluble vitamins: niacinamide, pyridoxine, thiamine, and riboflavin. The four vitamins are assayed simultaneously in multivitamin products not containing minerals. Thiamine currently is not quantitated in formulations containing minerals because it is not stable under the extraction conditions. The method was applied to the analysis of at least 12 different multivitamin products, including various formulations of sterile products, fluids, compressed tablets, and coated, compressed tablets. The method is stability indicating and is applicable to single-tablet assays.

Keyphrases □ Niacinamide—simultaneous high-pressure liquid chromatographic analysis with pyridoxine, riboflavin, and thiamine in multivitamin products □ Pyridoxine—simultaneous high-pressure liquid chromatographic analysis with niacinamide, riboflavin, and thiamine in multivitamin products □ Riboflavin—simultaneous high-pressure liquid chromatographic analysis with niacinamide, pyridoxine, and thiamine in multivitamin products □ Thiamine—simultaneous high-pressure liquid chromatographic analysis with niacinamide, pyridoxine, and riboflavin in multivitamin products □ Vitamins—niacinamide, pyridoxine, riboflavin, and thiamine, simultaneous high-pressure liquid chromatographic analysis in multivitamin products □ High-pressure liquid chromatography—analysis, niacinamide, pyridoxine, riboflavin, and thiamine in multivitamin products

A substantial amount of information has been published since 1970 on the use of high-pressure liquid chromatography (HPLC) for the analysis of water-soluble vitamins. An excellent review of the subject was prepared by Wittmer and Haney (1). Many of the alternative chemical and microbiological assay methods have time-consuming sample preparations and are not specific. HPLC is the preferred method for vitamin analysis, especially when the sample preparation is simple.

A recent report described the simultaneous determination of niacinamide, pyridoxine, thiamine, and riboflavin (2). Both the previously reported method and the method described in this paper use a paired-ion mobile phase system with a reversed-phase column (3).

The HPLC procedure described in this report has been used for routine analysis for 3 years. The method has been applied to 12 different multivitamin formulations, and work is continuing to add more applications. An average

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