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# SYNTHESIS OF NOVEL GLUCURONIDE CONJUGATES OF RETINOID CARBOXYLIC ACIDS

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ABSTRACT: The synthesis and characterization of the novel glucuronide conjugates of 9-cis and 13-cis retinoic acid and of all-trans, 9-cis and 13-cis 4-oxoretinoic acid are described.

Vitamin A is required for several essential life processes, including growth, cell differentiation, reproduction and vision[1]. Several geometric isomers of retinoic acid (RA), which is a derivative of vitamin A (retinol), are found endogenously in blood and other tissues of humans and other animals[1]. The role of retinoids in gene expression is mediated primarily by the interaction of RA with two families of nuclear retinoic acid receptors: RAR and RXR. 9-cis Retinoic acid is a specific ligand for RXR, whereas either 9-cis or all-trans RA activate the RAR family[1]. Thus, retinoid isomerization plays a key role in cell differentiation as it does in vision[2]. All-trans retinoic acid (tRA ) (**1**; R = **a**), 13-cis retinoic acid (13cRA)(**1**; R = **b**) as well as other retinoids are clinicaly useful in the treatment of dermatological disorders[1] and certain types of cancer[1]. All isomers do not show the

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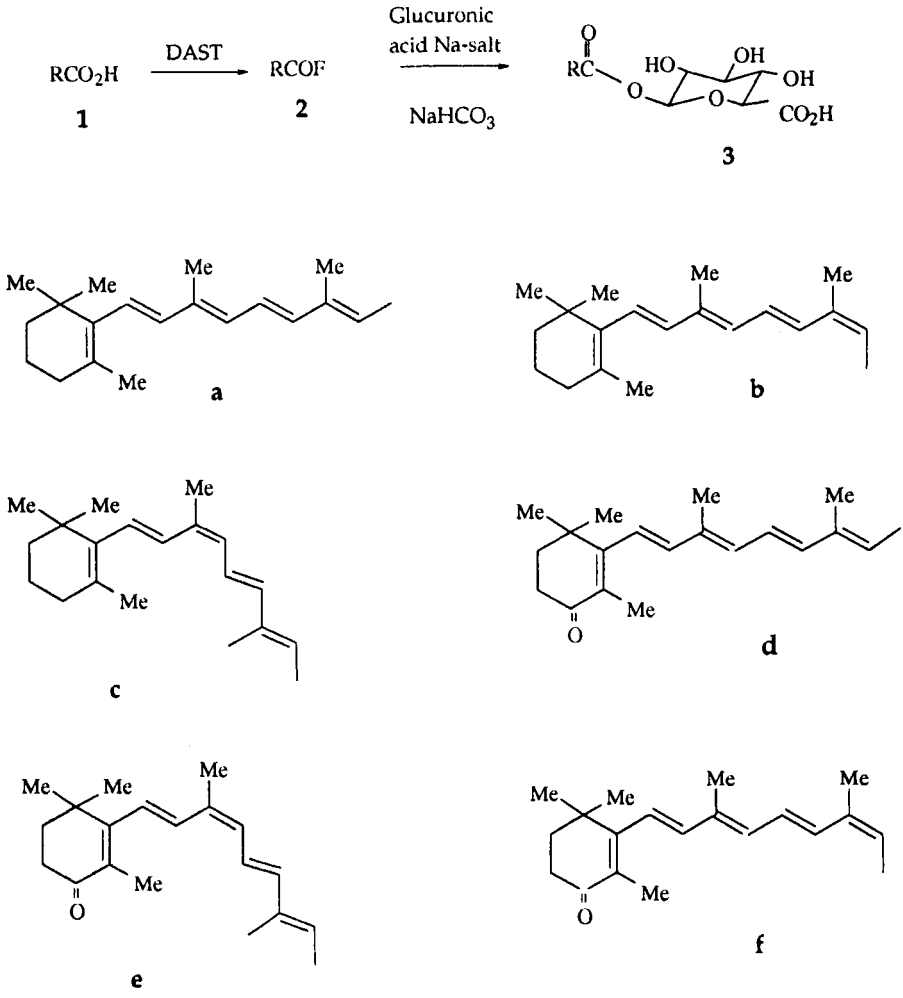
same biological activity; e.g., tRA is more toxic and teratogenic than 13cRA[1]. 9-cis Retinoic acid (9cRA) (**1**; R = **c**) is more potent[3] than tRA in inducing pattern duplication in the developing chick wing bud.

4-Oxoretinoic acid[4], 4-hydroxyretinoic acid[4c], 5,6-epoxyretinoic acid[4c,5] retinoyl ß-glucuronide[5a,6] are common biliary or plasma metabolites of RA. Glucuronides of 4-oxoretinoic acid[7a,8], 13cRA[9] and, more recently, 9cRA[10] have also been identified. Because of the nonavailability of reference compounds, the identity of these glucuronide conjugates, except for all-trans retinoyl ß-glucuronide (tRAG) (3; R = **a**), has been based on the cleavage of the conjugate to the corresponding retinoid carboxylic acid on treatment with ß-glucuronidase[7-10]. By use of synthetic tRAG[11], it has been demonstrated that tRAG is biologically as active[12], but much less toxic[12] and teratogenic[13] than tRA. Like tRA, tRAG is also efficacious for the topical treatment of human acne[14], but unlike tRA, tRAG is nonirritating to the human skin[14]. The biological activity and toxicity of the other glucuronide conjugates of RA and oxoRA are not known. Information on the synthesis and chemical properties of the glucuronide conjugates of the isomers of retinoic acid and of 4-oxoretinoic acid should, therefore, be useful to investigators in the retinoid field.

In this paper, we describe the synthesis (Scheme 1) and characterization of 13-cis retinoyl ß-glucuronide (**3**; R = **b**), 9-cis retinoyl ß-glucuronide (**3**; R = **c**), all-trans 4-oxoretinoyl ß-glucuronide (**3**; R = **d**), 9-cis 4-oxoretinoyl ß-glucuronide (**3**; R = **e**) and 13-cis 4-oxoretinoyl ß-glucuronide (**3**; R = **f**).

## RESULTS AND DISCUSSION

The glucuronide conjugates of retinoid carboxylic acids were synthesized[11] according to Scheme 1. Retinoid carboxylic acid (**1**) (2 mmol) was




**Scheme 1.** Synthesis of glucuronide conjugates of retinoid carboxylic acids

reacted with diethylaminosulfur trifluoride (DAST) (2.1 mmol) in anhydrous diethyl ether at -70° C to give the corresponding retinoyl fluoride (**2**) in almost quantitative yields. No significant isomerization of any of the cis-trans isomers of the retinoids studied in this work was observed. It is necessary to purify **2** on a

short column of silica before allowing it to react with glucuronic acid, inasmuch as the crude product of fluorination contained by-products that prevented the glucuronidation reaction to proceed. **2** (2 mmol) dissolved in acetone (2 vol) was stirred at room temp. with the sodium salt of glucuronic acid (6 mmol) dissolved in water (1 vol) in the presence of sodium bicarbonate. The corresponding retinoyl ß-glucuronide (**3**) was formed in 25-50% yield. It is necessary to purify **3** by column chromatography on silica as described earlier[11] to remove unreacted **2**, some **1** that resulted from hydrolysis of **2**, and traces of α-anomer of **3**. The purity of each retinoyl glucuronide was determined by reversed-phase HPLC as described earlier[11] on a Waters 5 μ "Resolve" 15-cm column or on a Rainin 3 μ Microsorb-MV 10-cm column by using solvent mixtures of varying composition of methanol and water containing 10mM ammonium acetate.

The identity of the retinoyl glucuronides (**3**) was based on their characteristic UV-visible and $^1$H-NMR spectra. The UV-visible absorption spectral data recorded on a Shimadzu model UV-240 or UV-2101 PC spectrophotometer are shown in Table 1.

**Table 1**

UV-visible Absorption Spectral Data of Retinoyl Glucuronides

| Isomer | Retinoyl ß-glucuronide (λmax, nm) | Solvent | 4-Oxoretinoyl ß-glucuronide (λmax, nm) |
|---|---|---|---|
| all-trans | 358 | Methanol | 364, 289 |
| | 365 | Water | 374, 293 |
| 13-cis | 369 | Methanol | 367, 288 |
| | 377 | Water | 377, 296 |
| 9-cis | 353, ~ 275 | Methanol | 356, 286 |
| | 363, ~ 280 | Water | 365, 295 |

The $^1$H-NMR spectra recorded in $CD_3OD$ on a Varian model XL400 NMR spectrometer (400 MHz) are shown in Table 2.

Further confirmation of the ß-glucuronide structure of the retinoid conjugates was done by enzymatic hydrolysis of the glucuronide conjugates.



**Table 2**

$^1$H-NMR Spectral Data (δ ppm)$^+$ of Retinoid Glucuronides

| | Retinoyl glucuronide | | | 4-Oxoretinoyl glucuronide | | |
|---|---|---|---|---|---|---|
| | at | 13-cis | 9-cis | at | 13-cis | 9-cis |
| C1 $(Me)_2$ | 1.03 | 1.03 | 1.04 | 1.21 | 1.21 | 1.21 (s)* |
| C2 $(CH_2)$ | 1.50 | 1.50 | 1.49 | 1.88 | 1.87 | 1.88 (m,t)* |
| C3 $(CH_2)$ | 1.65 | 1.65 | 1.65 | 2.50 | 2.53 | 2.54 (m,t) |
| C4 $(CH_2)$ | 2.03 | 2.06 | 2.05 | - | - | - |
| C5 (Me) | 1.71 | 1.71 | 1.74 | 1.82 | 1.83 | 1.86 (s) |
| C7 (CH) | 6.32 | 6.26 | 6.72 | 6.45 | 6.45 | 7.0 (d) |
| C8 (CH) | 6.17 | 6.17 | 6.32 | 6.45 | 6.45 | 6.42 (d) |
| C9 (Me) | 2.01 | 2.01 | 2.0 | 2.06 | 2.06 | 2.06 (s) |
| C10 (CH) | 6.2 | 6.22 | 6.15 | 6.30 | 6.35 | 6.30 (d) |
| C11 (CH) | 7.12 | 7.05 | 7.10 | 7.14 | 7.15 | 7.19 (dd) |
| C12 (CH) | 6.40 | 7.76 | 6.32 | 6.50 | 7.83 | 6.45 (d) |
| C13 (Me) | 2.36 | 2.11 | 2.33 | 2.34 | 2.13 | 2.38 (d) |
| C14 (CH) | 5.87 | 5.73 | 5.86 | 5.91 | 5.78 | 5.90 (s) |
| C1' (H) | 5.53 | 5.53 | 5.70 | 5.53 | 5.52 | 5.52 (d) |
| C2'-5' (H) | ---------- 3.4 - 3.8 (m) ---------- | | | | | |

*s = singlet; d = doublet; t = triplet; m = multiplet. +Reference: tetramethyl silane

Incubation of the retinoyl ß-glucuronide with ß-glucuronidase at 37°C in phosphate buffer at pH 7.4 resulted in the corresponding retinoid carboxylic acid.

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