Development of aqua-soluble compounds based on vitamin A series molecules: Synthetic transformations of β-ionone

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Several β-ionyl compounds bearing hydrophilic moieties like a carbohydrate, amine, phosphate and amino acid have been prepared and characterized. The solubilisation behaviour of these compounds has been examined by studying their UV-Vis absorption behaviour in micelles.

Keywords: β-Ionone, radioprotective agents, vitamin A compounds

Carotenoids and retinoids, particularly compounds in vitamin A series are involved in a wide range of life-sustaining biological processes like vision, light-induced proton transport, cell differentiation and growth, prevention of oxidative stress, regulation of the immune system and many others. The hydrophobic nature of these compounds has, however, been an impediment in some of their applications. Reported attempts to obviate this problem include liposomal encapsulation and attachment of solubilizing groups to them. trans-4-hydroxy-β-ionone 1b was synthesized en route to further elaboration, along with the formoxy compound 1c as a side product, by a three-step sequence involving allylic bromination of 1a followed by nucleophilic substitution with the formate ion and subsequent alkaline hydrolysis. It may be mentioned here that 1b was also prepared efficiently in a yield of 46% by subjecting the bromo-compound formed in the first step of the above sequence directly to a nucleophilic displacement reaction with water at RT thus not only avoiding the need to use the HCO2Na/HCO2H/Na2CO3 combination but also eliminating the additional task of purifying 1b from the formate ester 1c.

α-α-D-Gluco-conjugate 1d was synthesized from 1b and 2,3,4,6-tetra-O-benzoyl-α-glucopyranosyl bromide by a Koenigs-Knorr reaction as described elsewhere. The 1H NMR spectrum of this product distinctly exhibited signals that are ascribable to its sugar and aglycone portions. The doublet centered at δ 6.86 with a J-value of 3.6 Hz is assigned to the anomeric hydrogen. The magnitude of the J-value is suggestive of equatorial disposition for this hydrogen, a claim further corroborated by an absorption peak at 855 cm⁻¹ in the IR spectrum of the conjugate. The 13C NMR spectrum of 1d also accords quite well with the structure shown. The ester carbonyl carbon atoms are huddled together in the range δ 166.14-164.46, as are...
Figure 1 — Chemical structures of synthesized β-ionyl compounds 1b–1g

five of the carbons of the glucose portion in the region δ 70.60-62.58, with the anomeric carbon appearing at δ 90.15. Furthermore, the 19 13C-signals in the range δ 133.96-128.44 together with an additional absorption peak at δ 140.53 are reflective of the twenty distinct sp² (olefinic and aromatic) carbon atoms possessed by 1d. While the signal due to C-4 of the ionyl portion of the conjugate is thought to be overlapping with the solvent (CDCl₃) peaks, that due to the carbonyl carbon atom (C-9) has apparently not been discernible, though its presence is readily elicited from a singlet at δ 2.35 (C-10 hydrogens) and an absorption peak appearing as a shoulder at 1675 cm⁻¹ in the 1H NMR and infrared spectra, respectively, of the compound. It is noteworthy that a carbonyl carbon atom eluding detection by 13C NMR spectroscopy is not unprecedented 12. The mass spectrum of 1d did not show an ion peak at m/z 786 expected for the molecular ion [C₄₃H₄₆O₁₁]+. It did display, however, a base peak at m/z 579.1 ascribable to the sugar portion resulting from fracture of the parent molecule at the glycosidic bond as well as peaks at m/z 207, 189.1 and 108.1 that can reasonably be associated with fragment ions emanating from the aglycone part of the molecule.

In a further endeavour, 1b was also allowed to undergo, in separate experiments, a DCC promoted esterification 19 reaction with N-Boc-L-valine and phosphorylation 20 with triethyl phosphite to afford, respectively, amino acid ester 1e and phosphate ester 1f. The former conjugate was obtained as a mixture of stereoisomers as suggested by some closely spaced peaks in its ¹H and ¹³C NMR spectral data. Resonance lines of protons on the ionyl portion of 1e are observed at δ 7.18 (d, J =16.4 Hz, H-7), 6.13 (d, J =16.4 Hz, H-8), 4.23 (m, H-4), 2.32 (s, H-10), 1.71 (s, H-13), and 1.05, 1.09 (s, s, H-11, H-12). Likewise, the protons of the Boc-valine moiety of 1e are revealed at δ 1.45 (s, t-Bu), 1.00 and 0.98 (d, d, J =2.4 and 2.8 Hz, CH(CH₃)₂), 5.03 (d, J =8.8 Hz, NH), and 5.26 (m, CHNH). In conformity with the structure shown for 1e, its ¹³C NMR spectrum displayed signals, amongst others, at δ 198.14, 172.24(d), 156.67, 155.74, 142.06(t), 133.46, 129.31(d), 79.98(d), 72.94(d) and 58.84(t). The mass spectrum of 1e displayed a pseudo-molecular ion peak at m/z 430.2 (M⁺ + Na).

The spectral data obtained for 1f are also in line with the structure shown. Seventeen resonance lines in its ¹³C NMR spectrum included peaks at δ 198.61, 142.87, 139.47, 134.21, 133.21, 70.00, 63.82 and 63.77. All the hydrogens of phosphate 1f are also fully accounted for by its ¹H NMR spectrum which exhibited, inter alia, peaks at δ 7.20 (d, J =16.4 Hz, H-7), 6.13 (d, J =16.4 Hz, H-8), 4.15 (m, 2×OCH₂), 4.02 (t, J =4.4 Hz, H-4), 2.31 (s, H-10), 1.85 (s, H-13), 1.35 (t, OCH₂CH₂), and 1.08, 1.05 (s, s, H-11, H-12). The appearance of a single peak at δ -2.70 in ³¹P NMR
spectrum confirmed incorporation of phosphate moiety in the conjugate. Lack of a method for the selective removal of the ethyl groups precluded conversion of 1f into the corresponding phosphoric acid derivative. An alternative was thus sought in an Arbuzov type reaction at RT of trans-4-bromo-β-ionone21 and triethyl phosphate with the intention of getting the corresponding phosphonate. However, the reaction led to a product having the same spectral characteristics as 1f. A further attempt to introduce a phosphoric acid moiety at C-4 of 1a via phosphorylation of 1b with phosphorous oxychloride followed by hydrolysis of the reaction mixture did not yield the anticipated compound. The product isolated from this reaction turned out to be 4-oxo-trans-β-ionone.

In a further attempt to functionalize C-4 of 1a, trans-4-bromo-β-ionone21 was allowed to undergo nucleophilic substitution reaction with guanidinoacetic acid to afford imino-ester 1g. The 1H NMR spectrum of 1g bore signals at δ 7.17 (d, J=16.4 Hz, H-7), 6.03 (d, J=16.4 Hz, H-8), 4.02 (q, J=7.2 Hz, OCH2CCH2NH), 3.92 (t, J=4.8 Hz, H-4), 2.22 (s, H-10), 1.75 (s, H-13), 1.16 (t, J=6.8, 7.6 Hz, CH2NH), 0.98 and 0.95 (s, s, H-11, H-12). The three terminal imino hydrogens (C(NH)NH2) have apparently coalesced into a singlet at δ 1.95. Included in the 13C NMR spectrum of 1g are signals located at δ 198.99, 173.61, 171.38, 69.53 and 60.47 whilst a strong peak at 3420 cm⁻¹ in its IR spectrum is assertive of the NH groupings. The mass spectrum of 1g exhibited, among others, an ion peak at m/z 263.1 (M⁺-44). An analogous attempt to attach ascorbic acid to C-4 of 1a via a nucleophilic substitution reaction of an aqueous solution of sodium L-ascorbate with trans-4-bromo-β-ionone21 was unsuccessful. The reaction yielded, instead, alcohol 1b along with an unidentified material.

To get an idea about solubility, localisation and translocation of these compounds through biological membranes, the UV-Vis absorption spectra of these compounds have been examined in membrane mimetic system of micelles. The λ_max of 1a in reverse micelle of sodium bis(2-ethylhexyl) sulphosuccinate (AOT) is the same as that in the hydrocarbon solvent n-heptane (Table I and Table II). However, polar environment causes red shift in its λ_max. In normal micelle of cetyltrimethyl ammonium bromide (CTAB), it gets anchored in relatively polar environment. On the other hand, the λ_max of hydroxy ketone 1b and phosphate ester 1f shifted slightly to a longer wavelength (278 nm) in the AOT reverse micelle with ω = 6, 8, 10, 12 relative to their λ_max value of 275/276 nm in the non-polar solvent n-heptane (Table I). The imino ester 1g likewise experienced a red shift of 2 nm in its λ_max value from 276 nm in n-heptane (Table I) to 278 nm in reverse micelle of ω = 10, 12 (Table II).

Based on the absorption data in Tables I and II, it can be said that while 1a is completely engulfed by the bulk organic solvent of the AOT/hexane/water system, compounds 1b, 1f, and 1g are embedded in the interfacial region of the reverse micelle with their polar groups oriented towards the water pool. The absence of any discernible absorption maximum for gluco-conjugate 1d and amino acid ester 1e in either micelle [CTAB (aq)] or H2O (Table I) lends itself to suggest that these derivatives are apparently more hydrophobic than the parent ketone 1a. Attempts to debenzoylate 1d with ammonia gas have proved unsuccessful. These observations are interpreted in terms of slightly better aqua-solubility of 1b, 1f and 1g relative to the parent compound 1a, a result ascribable to the solubilising effect of the polar moieties these derivatives bear.

In conclusion, β-ionyl structure has been further functionalized leading to several interesting compounds with altered hydrophilicity. Thus, this work gives a new direction towards developing aqua-soluble retinoids with potentially useful biological properties like antioxidant and radioprotection.

Experimental Section

All the chemicals and solvents used in this work were procured from suppliers in Mumbai, India. Solvents used were purified and dried following standard procedures. Petroleum ether used was of 60-80°C range. 1H and 13C NMR spectra were recorded with a Varian VXR 400 MHz FTNMR instrument using TMS as internal standard. Mass spectra were recorded on a Micromass Q-TOF micro instrument. FTIR spectra were recorded with Nicolet Impact-400 series spectrophotometer. UV-Vis absorption spectra were recorded on a Shimadzu UV-160A or JASCO V-570 UV/VIS/NIR spectrophotometer.

1.0 × 10⁻² M CTAB normal micelles were prepared by dissolving CTAB in distilled water. β-Ionyl compounds were added to this solution, which was further stirred for a few minutes to obtain a clear
Table I — UV-Vis absorption data of 1a, 1b, 1d-1g in CTAB micelle and solvents of varying polarity

<table>
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<th>Compd</th>
<th>Solvent</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Compd</th>
<th>Solvent</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Compd</th>
<th>Solvent</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
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<td>282</td>
<td>1d</td>
<td>n-Heptane</td>
<td>276</td>
<td>1f</td>
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<td>Acetonitrile</td>
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<td></td>
<td>Water</td>
<td>hs</td>
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<td>Water</td>
<td>286</td>
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<sup>a</sup>[CTAB] = 1.0 × 10<sup>-2</sup> M. (hs: hardly soluble).

Table II — UV-Vis absorption data of 1a, 1b, and 1d-1g in AOT reverse micelles

<table>
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<tr>
<th>Compd</th>
<th>Solvent&lt;sup&gt;b&lt;/sup&gt; ω = [H&lt;sub&gt;2&lt;/sub&gt;O]/[AOT]</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; nm</th>
<th>Compd</th>
<th>Solvent&lt;sup&gt;b&lt;/sup&gt; ω = [H&lt;sub&gt;2&lt;/sub&gt;O]/[AOT]</th>
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<tr>
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<td>2-12</td>
<td>276-278</td>
<td>1f</td>
<td>2-12</td>
<td>276-278</td>
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<tr>
<td>1d</td>
<td>2-12</td>
<td>272-274</td>
<td>1g</td>
<td>2-12</td>
<td>276-278</td>
</tr>
</tbody>
</table>

<sup>b</sup>AOT/n-hexane (1.0 × 10<sup>-2</sup> M) reverse micelle of varying hydration degree (ω, molar ratio of [H<sub>2</sub>O]/[AOT]).

For reverse micellisation, the β-ionyl compound was added to n-hexane solution of AOT (1.0 × 10<sup>-2</sup> M) containing water required for a specific ω (molar ratio of [H<sub>2</sub>O]/[AOT]). The mixture was carefully hand-shaken to obtain a clear solution for absorption studies. The final concentration of the β-ionyl compound was maintained at 1.0 × 10<sup>-5</sup> M.

4-Hydroxy-β-ionone, 1b

1b was prepared by following the reported procedure<sup>14</sup>. In addition, it is found that it can also be prepared in 46% yield by subjecting the bromo-compound formed in the first step of the reported procedure directly to a nucleophilic displacement reaction with water at RT thus not only avoiding the need to use the HCO<sub>2</sub>Na/HCO<sub>2</sub>H/Na<sub>2</sub>CO<sub>3</sub> combination but also eliminating the additional task of purifying 1b from the formate ester 1c. In the new procedure, to a warm solution of 1a (1.2 g, 6 mmol) in carbon tetrachloride (20 mL) was added freshly purified N-bromosuccinimide (1.25 g, 7 mmol) and catalytic amount of benzylo peroxide. After refluxing for 1.5 hr, the mixture was cooled to 0°C and petroleum ether (10 mL) was added to it. The reaction mixture was then filtered and most of the solvents removed under reduced pressure. The contents of the flask were then taken up in water containing little 1,4-dioxane and stirred overnight at RT. Usual work-up involving extraction of organic matter with dichloromethane, drying with anhydrous sodium sulphate followed by removal of dichloromethane under reduced pressure yielded dark brown oil. Column chromatography (silica gel, 10% ethyl acetate-petroleum ether) of the dark brown oil afforded 1b in 46% yield (0.53 g) as light brown oil. The compound so obtained exhibited spectral data (IR, <sup>1</sup>H NMR) as reported earlier<sup>13,22</sup>. In addition, it showed the following <sup>13</sup>C NMR (CDCl<sub>3</sub>) data: δ 198.81, 143.07, 139.33, 134.40, 133.11, 69.92, 34.82, 34.76, 28.93, 28.40, 27.66, 27.45, 18.62.

(E)-2-(Benzoyloxy methyl)-6-(2,4,4-trimethyl-3-(3-oxobut-1-enyl)cyclohex-2-enyloxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate, 1d

A mixture of 2,3,4,6-tetra-O-benzoyl-D-glucopyranosyl bromide (0.73 g, 1.11 mmol) prepared as described elsewhere<sup>15</sup> and 1b (0.25 g, 1.2 mmol) in dry acetonitrile (25 mL) was subjected to a mercuric bromide (0.36 g, 1.0 mmol) catalysed Koenigs-Knorr
coupling reaction following a literature procedure. Purification of the crude reaction mixture by silica gel column chromatography using 10% ethyl acetate in petroleum ether as eluent afforded 63 mg (7%) of conjugate 1d as a dark brown solid, m.p. 140°C (dec.); IR (KBr): 2959, 2926, 2867, 1731, 1675, 1270, 1178, 1163, 1122 cm⁻¹; ¹H NMR (CDCl₃): δ 1.19, 1.15 (s, s, 6H, H-11 and H-12), 1.80 (s, 3H, H-13), 2.54, 1.83 (m, 4H, H-3 and H-2), 2.35 (s, 3H, H-10), 4.47-4.51 (m, 1H, H-6'), 4.61-4.66 (m, 2H, H-5', H-6'), 5.69 (dd, 1H, H-2'), 5.88 (t, J = 10.0, 9.6 Hz, 1H, H-4'), 6.19 (d, J = 16.4 Hz, 1H, H-8), 6.33 (t, J = 10.0 Hz, 1H, H-3'), 6.86 (d, J = 3.6 Hz, 1H, H-1'), 8.18-7.26 (m, Ar-protons and H-7); ¹³C NMR (CDCl₃): δ 26.80, 27.39, 29.76, 34.24, 34.27, 37.43, 42.35, 62.58, 68.99, 70.56, 70.57, 70.60, 76.57, 90.15, 128.44, 128.47, 128.50, 128.68, 128.83, 128.86, 128.95, 129.11, 129.66, 129.81, 129.86, 129.91, 129.96, 130.09, 133.17, 133.40, 133.53, 133.57, 133.96, 140.53, 164.46, 165.21, 165.42, 165.98, 166.14; MS: m/z (%) 189.1 (13), 207 (9), 231.0 (39), 579.1 (100).

(E)-2,4,4-Trimethyl-3-(3-oxobut-1-enyl)cyclohex-2-enyl methylbutanoate, 1e

Compound 1e was prepared following a literature procedure for esterification reactions. A solution of N-Boc-L-valine (0.5 g, 2.3 mmol), N,N-dicyclohexylcarbodiimide (0.5 g, 2.4 mmol), dicyclohexylcarbodiimide (0.5 g, 2.4 mmol), triethylphosphite (0.15 g, 0.92 mmol) in a yield of 0.5 g (20%). IR (CHCl₃): 2940, 2869, 1673, 1367, 1301, 1295, 1287, 1270, 1178, 1163, 1122 cm⁻¹; ¹H NMR (CDCl₃): δ 1.08, 1.05 (s, s, 6H, H-11 and H-12), 1.35 (t, 6H, 2 × OCH₂CH₂), 1.45, 1.70, 1.92 (m, m, m, m, m, m), 2.05, 2.06 (m, m, m, m, m, m), 2.26 (s, 3H, H-10), 3.92 (t, J =4.8 Hz, 1H, H-4), 4.02 (q, J = 7.2 Hz, 2H, CH₂NH), 6.03 (d, J=16.4 Hz, 1H, H-8), 7.17 (d, J=16.4 Hz, 1H, H-7); ¹³C NMR (CDCl₃): δ 18.48, 27.19, 27.65, 28.24, 28.72, 34.57, 34.86, 60.47, 69.53, 132.79, 134.96, 138.80, 143.36, 171.38, 173.61, 198.99; MS: m/z (%) 107.1 (36), 135.1 (100), 191.2 (12), 231.2 (9.6), 263.1 (1.2) [M⁺-44].

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References
5  Barua A B & Olson J A, Biochem J, 244, 1987, 231.