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POLYMER BOUND PYRROLE COMPOUNDS. REVERSIBLE ANCHORING OF BILIRUBIN AND BILIVERDIN TO A POLYSTYRENE MATRIX

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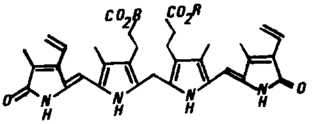
Abstract: Both bilirubin and biliverdin, via their cesium salts, have been covalently attached to an insoluble polystyrene matrix (the so-called Nbb-resin), some chemical transformations have been carried out upon the supported pigments, and the resulting products have been detached by alkaline methanol .

The binding of bile pigments to polymers through appropriate covalent linkages should supply good models for the naturally occurring biliproteins (proteins to which a bile pigment is covalently bound). Most of the related work previously published is concerned with non-covalent protein-bilirubin interactions[1]. A few papers have appeared involving tetrapyrroles covalently bound to chloromethylated polystyrene[2], but these refer to macrocyclic structures. To our knowledge, no previous report on polymer-based linear tetrapyrroles has appeared. In the following we describe the preparation, i.r. spectroscopy, a few synthetic applications, and cleavage of insoluble polymer-bound bilirubin and biliverdin.

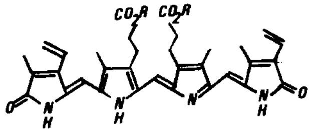
The cesium salt of bilirubin (3) was prepared by suspending the rubin free acid in dimethylformamide (1 mg/mL) followed by addition of the same number of equivalents of Cs_2CO_3 (aqueous, 0.04 M) and by vacuum evaporation of the solvent to dryness. Covalent anchoring of this cesium salt to chloromethylcopoly(styrene-1%-divinylbenzene)[3] to give compound 1 proceeded only with low yields (ca. 10%). The more reactive bromomethyl-Nbb-resin[4] provided a better alternative to the standard Merrifield polymer. Reaction of 0.1 mmol of the cesium salt of 3 (Sigma) with 200 mg of bromomethyl-Nbb-resin (1.1 meq of Br/g of polymer) in dry, amine-free dimethylformamide during 24 h at RT, in the absence of light, led to the polymer-bound bilirubin 2 with reasonable yield (55% assuming dianchoring; 0.3 mmol of pigment/g of polymer). The resulting deep-red resin, after washing with 10% $AcOH/CH_2Cl_2$ to protonate any free carboxylate residue, showed bands of bilirubin diester in the i.r. spectrum, at 3340 (N-H stretch) and 1740 cm^{-1} (ester C=O stretch), but not at hydrogen-bonded O-H frequencies[5]. In a parallel experiment, the cesium salt of biliverdin (7) was also bound to bromomethyl-Nbb-resin with similar yield. The i.r. spectrum of the resulting deep-blue product 6 again showed only bands due to the diester.

In order to cleave bilirubin from 1, prolonged treatment at RT with 1:1 mixtures of methanol and different tertiary amines was unsuccessfully attempted. However, when 1 in methanol containing ascorbic acid and EDTA-disodium salt, was treated for several hours at RT with the same volume of a 2% (w/v) KOH in MeOH[6], some bilirubin dimethyl ester (4) was obtained after glycine-HCl buffer neutralisation of the filtered solution and subsequent $CHCl_3$ extraction. Almost quantitative cleavage of the pigment from 1 and from 2 was achieved by brief (2x3 min) treatment with MeOH:Dioxane:4N-NaOH (30:9:1; 2 mL per 100 mg of resin) at RT[7]. The solution was neutralised with glycine-HCl, and 4 was obtained by CHCl$_3$ extraction. The product was reddish-brown in colour, and was identified by TLC and by ^{1}H-n.m.r. spectroscopy by comparison with an authentic sample[6]. Exclusive isolation of 4 indicates that bilirubin was doubly attached to the resin in 1 and 2 (through its two propionic side chains). Identical base treatment of the polymer-bound biliverdin 6 led to isolation of mostly the dimethyl ester (8) (less than 10% of the monomethyl ester (9) was formed). The product was blue, and was identified as biliverdin by TLC and ^{1}H-n.m.r. spectroscopy, by comparison with an authentic sample[8].

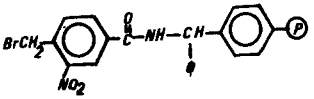
In order to check the synthetic possibilities of the polymer-bound linear tetrapyrroles, two reactions were carried out: i) DDQ oxidation of polymer-bound bilirubin 2 to polymer-bound biliverdin 6[9], and ii) the NaBH$_4$ reduction of 6 to 2[10]. When a slurry of 2 in dimethyl sulphoxide was treated with a two-fold molar excess of DDQ during 10 min at RT with Vortex-mixing, the red colour progressively became blue, and subsequent cleavage of the pigment by base yielded exclusively 8. Similarly, treatment of 6 in MeOH:Dioxane (1:1) with a ten-fold molar excess of NaBH$_4$ during 1 min at RT with Vortex-mixing, afforded polymer-bound bilirubin 2, as proven by subsequent detachment of only the dimethyl ester 4. With higher hydride concentration, simultaneous alkaline cleavage of the pigment occurred .



R= CH$_2$-copoly(styrene-1%-divinylbenzene) (1);
R= CH$_2$-Nbb-resin (2); R= H (3); R= CH$_3$ (4).



R= CH$_2$-copoly(styrene-1%-divinylbenzene) (5);
R= CH$_2$-Nbb-resin (6); R= H (7); R= CH$_3$ (8);
R= H, R= CH$_3$ (9) .



Bromomethyl-Nbb-resin .

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