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Acid-Catalyzed Addition of Alcohols and Thiols to Bilirubin

Recently we found that bilirubin (I) reacts under UV-light with alcohols[1] and more readily with thiols[2] to give adducts such as II and III in moderate and high yields respectively. On this basis we hypothesized that at least part of the serum bilirubin in animals and humans irradiated with blue-violet light (for instance, in jaundiced newborn infants during phototherapy[3]) may be eliminated as photoadducts with nucleophilic substances occurring in the body. In agreement with this hypothesis recent studies on the photocatabolism of bilirubin in the congenitally jaundiced (Gunn) rat[4], as well as in children with Crigler-Najjar syndrome[5], indicate that irradiation causes a marked increase in the biliary excretion of bilirubin derivatives, in large part yellow, diazopositive and water-soluble. However, these photocatabolites qualitatively resemble those found under control lighting conditions, thus suggesting that exposure to light simply accelerates the catabolism of bilirubin by mechanisms similar to those normally operative in patients with Crigler-Najjar syndrome and in Gunn rats[4,5]. We now report taht in strongly acid media bilirubin undergoes regiospecific[6] addition of protic nucleophiles (ROH, RSH) affording the same products obtained by irradiation.

When bilirubin was dissolved in chloroform (1 mg/ml) containing 5% (v/v) methyl thioglycollate and a few crystals of *p*-toluensulfonic acid, disappearance of the starting pigment was complete in ca. 8 h (at r.t. in the dark) and accompanied by the formation of a compound migrating just as IV[2] in TLC[7]. This was then obtained pure (60% yield) after washing with methanol the residue of evaporation of the reaction mixture, and shown to be IV by comparison (UV, IR, NMR) with an authentic sample prepared by photoaddition.

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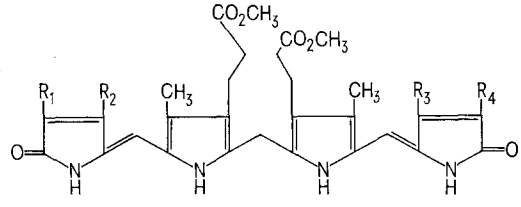
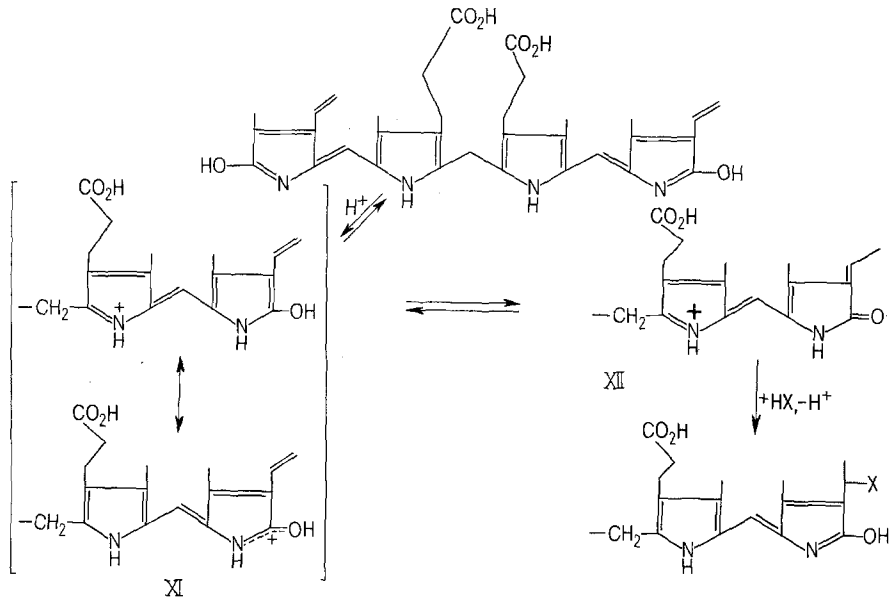
[2] P. MANITTO and D. MONTI, Experientia *28*, 379 (1972).
[3] See leading articles in *Bilirubin Metabolism in the Newborn* (Eds. D. BERGSMA, D. Y. Y. HSIA and C. JACKSON; The Williams and Wilkins Company, Baltimore 1970).
[4] J. D. OSTROW, J. clin. Invest. *50*, 707 (1971).
[5] E. W. CALLAHAN JR., M. M. THALER, M. KARON, K. BAUER and R. SCHMID, Pediatrics *46*, 841 (1970).
[6] This term is used here in an extension of its common usage. See S. TURNER, Chemistry in Britain, 1971, p. 191. – A. HASSNER, J. org. Chem. *33*, 2684 (1968).
[7] TLC was carried out on polyamide[2] (for VI) and on pre-coated silica gel plates (Merck) [solvent systems: methanol – benzene – chloroform 1.5:100:50 (v/v) for IV, V, VII, IX, X, XIII and XIV; idem 1:4.5:4.5 (v/v) for VIII; ethyl acetate – chloroform – ligroin 1:0.5:3 (v/v) for azopigments methyl esters]. All the new compounds gave correct elemental analyses.



	R_1	R_2	R_3	R_4
I	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$
II	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)OR$
III	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)SR$
IV	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)SCH_2COOCH_3$
V	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)OCH_3$
VI	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)SCH_2CH(NHCOCH_3)COOH$
VII	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)SCOCH_3$
VIII	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)SCH(CH_3)CONHCH_2COOH$
IX	$CH{=}CH_2$	CH_3	CH_3	$CH{=}CH_2$
X	CH_3	$CH{=}CH_2$	$CH{=}CH_2$	CH_3
XIII	$CH{=}CH_2$	CH_3	CH_3	$CH(CH_3)SCH_2COOCH_3$
XIV	$CH(CH_3)SCH_2COOCH_3$	CH_3	CH_3	$CH(CH_3)SCH_2COOCH_3$
XV	CH_3	$CH{=}CH_2$	CH_3	$CH(CH_3)OSO_3H$



Likewise, bilirubin in chloroform containing *p*-TsOH and 1. methanol (10%, v/v), or 2. N-acetyl-L-cysteine (2 mg/ml), or 3. thioacetic acid (5%, v/v), or 4. α-mercaptopropionylglycine (2 mg/ml) gave[7]: 1. V (7 days, 25% yield)[1], 2. VI (6 h, 80%)[2], 3. VII [6 h, 85%, NMR (DMSOd_6)[8] 1.54$_d$ (3H, J = 7 Hz, -CH_3), 2.33$_s$ (3H, CH_3COS), 4.66$_q$ (1H, J = 7 Hz, >CH-S-) and the ABX system of the *endo*-vinyl group between 5.5–7.2; $\lambda_{max}^{CHCl_3}$ 450 nm (ε 52,300)], 4. VIII [5 h, 87%, NMR(DMSOd_6) 1.27$_d$ (3H, J = 7 Hz, CH_3-CH-CO), 1.55$_d$ (3H, J = 7 Hz, CH_3-CH-S), 3.85$_d$ (-CH_2-NH-) partly buried beneath the broad singlet of the central methylene bridge at 4.10, and the ABX system of the *endo*-vinyl group between 5.5–7.3; $\lambda_{max}^{CHCl_3}$ 451 nm (ε 51,100)]. No isomeric adducts were detected in the reaction mixtures; in addition, no significant isomeric scrambling of bilirubin IXα (I) leading to formation of bilirubin IIIα (IX) and bilirubin XIIIα (X)[9] was observed in TLC under the above conditons as well as in chloroform containing *p*-TsOH only.

The formation of such adducts as IV–VIII can be rationalized as an initial protonation of the isovinylneoxanthobilirubinic acid moiety of bilirubin (as in XI) followed by a rearrangement (with proton transfer) to yield the cation XII[11] which may be attacked by the nucleophile HX (X = OR or SR) occurring in the medium. That bilirubin exists in acid media partially in protonated forms (XI as well as the corresponding one with the proton attached to the vinylneoxynthobilirubinic acid moiety) is strongly supported by the UV-spectrum of the pigment in chloroform containing *p*-toluensulfonic acid. In fact, this solution exhibits two absorption maxima at 517 (ε 132,000 calc.)[12] and 453 nm, the former appearing consistent with the presence of protonated dipyrromethene derivatives[13]. Furthermore, the mechanism proposed accounts for the regiospecificity[6] of the acid-catalyzed additions to bilirubin as a consequence of an interaction between the enolic hydroxyl (or the protonated carbonyl) and the vinyl group which occur in close proximity in the isovinylneoxanthobilirubinic acid moiety only[14]. Confirming evidence for the difference in reactivity between *endo*- and *exo*-vinyl groups in a biladiene-*a,c* skeleton resulted from the behavior of IX and X under the conditions for an acid-catalyzed addition of methyl thioglycollate: two derivatives corresponding to XIII and XIV[15] were obtained from the former isomer, whereas none from the latter.

All these data raise the possibility that enzyme-catalyzed additions of nucleophiles to the *exo*-vinyl group of bilirubin via a cation such as XII occur in vivo, and suggest that structures XV and II (R = glycosyl radical) are the most likely candidates for natural bilirubin sulfate[17] and for the alkali-stable bilirubin conjugate detected by KUENZLE as azopigment B_I[19]. In addition, the above results might explain the finding that the vinyl/isovinyl isomer ratio of azopigments derived from human and rat bile is almost always in favour of the vinyl isomer[19,20].

[8] Chemical shifts are in parts per million (δ) from internal tetramethylsilane; *s*, singlet; *d*, doublet; *q*, quartet.

[9] Acid-catalyzed isomeric scrambling of bilirubin IXα has been recently reported[10]. We obtained IX and X in satisfactory yields by preparative TLC[7] of the isomeric mixture arizing from the treatment of bilirubin (100 mg) in DMSO (20 ml) with *p*-TsOH (0.5 g) for 1 min (at r.t. under He) followed by precipitation with H_2O.

[10] A. F. DONAGH and F. ASSISI, Chem. Commun. *1972*, 117.

[11] The same cation was postulated as a transient intermediate in photo-induced additions to bilirubin[1].

[12] The molecular extinction coefficient was calculated considering the decrease of the maximum at 453 nm as due to the protonation of one chromophore of bilirubin.

[13] L. G. S. BROKER, F. L. WHITE, R. H. SPRAGUE, S. G. DENT JR., and G. VAN ZANDT, Chem. Rev. *41*, 325 (1947). – W. RUDIGER, in *Fortschritte der Chemie organischer Naturforstoffe* (Springer Verlag, Wien 1971), p. 73.

[14] J. C. KOHLI, M. S. WADIA and P. S. KALSI, Experientia *28*, 131 (1971), for another example.

[15] These adducts were identified by a comparison (co-chromatography[7]) of their ethyl anthranilate azopigments methyl esters[16] with those of IV and X.

[16] K. P. M. HEIRWEGH, G. P. VAN HEES, P. LEROY, F. P. VAN ROY and F. H. JANSEN, Biochem. J. *120*, 877 (1970).

[17] B. A. NOIR, A. T. DE WALZ and E. A. RODRIGUEZ GARAY, Biochim. biophys. Acta *222*, 15 (1970) and references cited therein. By contrast, WATSON's synthetic bilirubin disulfate[18,19] results probably from Markownikoff addition of H_2SO_4 to both *exo*-vinyl groups of bilirubin IIIα (IX) arizing from isomeric scrambling[9] of natural bilirubin IXα (I) under the reaction conditions.

[18] C. H. GREGORY and C. J. WATSON, J. Lab. clin. Med., *60*, 17 (1962). – C. C. KUENZLE, Biochem. J. *119*, 395 (1970).

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This would result from the occurrence in vivo of bilirubin derivatives carrying a substituent on their isovinyl-neoxanthobilirubinic acid portion (as in II and III), thus causing a deficiency of the detectable isovinyl azopigments.

Riassunto. In mezzo fortemente acido la bilirubina reagisce con gli alcooli e con i tioli fornendo con alte rese prodotti di addizione identici a quelli ottenibili per via fotochimica in assenza di acidità.

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The Isolation of Dehydrodiisoeugenol from the Aril of *Myristica fragrans* Houtt[1]

Myristica fragrans Houtt. is the economically important member of the family Myristicaceae. The seed (nutmeg) and the aril (mace) are widely used as spices and have a long history of abuse in view of their reported narcotic properties[2,3]. Whilst terpenoids and fatty acids constitute the major classes of compounds found in this species, propenylphenyl ethers are also present in significant quantities[3-5]. We now wish to report the isolation of an example of a dimeric propenylphenol, the coumaran derivative, dehydrodiisoeugenol (I). This compound was isolated from the phenolic fraction of a cold petroleum ether extract of freshly ground mace, by column chromatography on silica gel [eluting solvent: benzene/hexanes (1:1)].

The dehydrodiisoeugenol (I), [mass spectrum, found: $M^{+\cdot}$ 326.1515, calculated for $C_{20}H_{22}O_4$ 326.1518, major peaks: 326 (100%), 311 (10%), 283 (6%), 202 (10%), 164 (6%), 151 (11%), 137 (18%), IR ν_{max} 3560 cm^{-1}. UV λ_{max} (EtOH) 275 nm] was obtained as white crystals m.p. 130–132° (from benzene-hexane), lit.[6]; m.p. 132–133°. The NMR-spectrum ($CDCl_3$) showed the following signals: τ 3.07–3.26, m, 5 aromatic H; 3.65, d, J = 15 Hz, 1, αH; 3,95, m, 1, βH; 4.40, s, 1, OH; 4.94, d, J = 9 Hz, 1, α'H; 6.13, s, 3, OCH_3; 6.15, s, 3, OCH_3; 6.58, m, 1, β'H; 8.15, d, J = 6 Hz, 3, γH's; 8.63, d, J = 7 Hz, 3, γ'H's. These values are in agreement with those reported by LUDWIG et al.[7], for dehydrodiisoeugenol which was prepared by oxidation of isoeugenol (II). The compound (I) was first prepared by COUSIN and HÉRISSEY[6] in 1908, its phenylcoumaran structure I proposed in 1933[8] and verified several years later[9,10]. The identification of the isolated compound as (I) was verified by formation of the acetyl derivative (m.p. 111–113°, lit.[11] m.p. 113-5°. [Mass spectrum; $M^{+\cdot}$ 368 (27%) major peaks, 326 (100%), 311 (10%), 202 (14%), 174 (5%), 164 (9%), 153 (23%), 151 (11%), 137 (14%), 91 (10%), 77 (9%), 43 (50%)].

Since isoeugenol has been reported in the essential oil of mace[11] and can be converted to dehydrodiisoeugenol by oxidation, it was therefore necessary to ensure that the dehydrodiisoeugenol isolated from mace was not an artifact formed by oxidation of isoeugenol during the extraction procedure. This was achieved by detection of I by two-dimensional thin layer chromatography [Brinkmann silica gel G plates, Rf in diethyl ether/cyclohexane (50:50) = 0.46; Rf in benzene/acetone (95:5) = 0.43] of the fresh petroleum ether extract. The identity of the thin layer spot was established by comparison of the Rf values in two solvent systems and the colour reactions (Fast Blue B and Anisaldehyde reagents) of this substance with those of a purified sample of (I), and further verified by determination of the mass spectrum of the material extracted from the appropriate spot on the plate.

Dehydrodiisoeugenol (I) has not previously been reported as a plant constituent, however the analogous compound, dehydrodiconiferol alcohol (III) has been isolated from spruce cambium sap (cf. Ref.[12]).

Dehydrodiconiferol alcohol has been proposed as an intermediate in the biosynthesis of lignin and it, as well as dehydrodiisoeugenol, has been used as a model for spectroscopic and degradative studies in lignin chemistry.

Résumé. On a pu isoler le déshydrodiisoeugénol de l'arille de *Myristica fragrans* Houtt. (fleur de muscade).

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[3] J. E. FORREST and R. A. HEACOCK, Lloydia, in press.
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