the category of radioactive substances as to the safety regulations of most countries concerning the transport, handling and use of open radioactive sources. This very problem is left to the attention and responsibility of the competent national as well as international authorities.

Résumé. Des mesures de radioactivité de nombreux échantillons d'eau lourde et de substances deutérées de provenances les plus diverses ont montré que les substances de teneur élevée en deutérium (>99%) présentent une radioactivité spécifique de l'ordre de 5 μCi par gramme de deutérium, due au tritium d'origine naturelle concentré en meme temps que le deutérium. Le phénomène ne se limite pas aux isotopes de l'hydrogène. Il est de caractère général et comporte d'importances conséquences théoriques et pratiques.

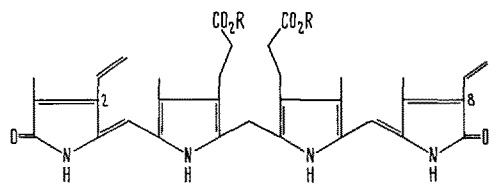
P. JORDAN and K. MAY

Laboratorium für organische Chemie der Eidg. Technischen Hochschule, Universitätsstrasse 6/8, CH-8006 Zürich (Switzerland), 9 June 1971.

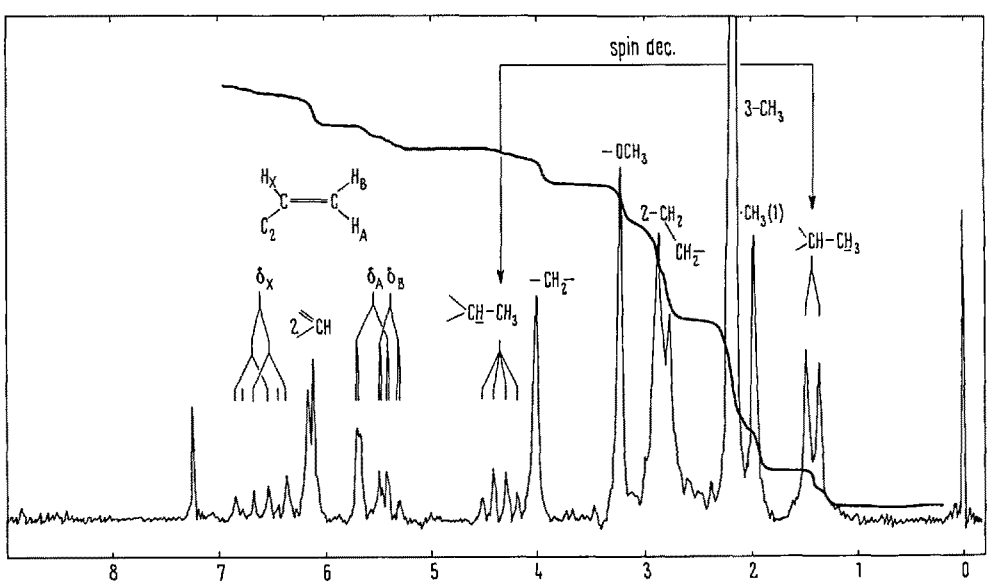
Photochemistry of Bilirubin

Although the phenomenon of a decrease in serum bilirubin levels in animals and humans irradiated with UV-light is well known (and exposure of infants to fluorescent light is commonly utilized to treat neonatal jaundice[1]), the mechanisms involved and the products of bilirubin decay have not been identified. In vitro photodecomposition of bilirubin was also studied at length[2], but no photoderivative has been isolated as yet. In this paper we wish to report the structure determination of the products we succeeded in isolating after irradiation of bilirubin in vitro in the presence of alcohols.

When bilirubin (I)[3] dissolved in chloroform containing 10% (v/v) methanol was exposed in a pyrex flask to a mercury lamp ($\lambda > 300$ nm)[4], formation of a photoproduct was observed and followed by TLC on polyamide[5] [methanol – 10% ammonia 9:1 (v/v)]. After 60–70% of bilirubin was converted (ca. 30 h), chromatography of the reaction mixture on a polyamide column developed with acetone gave the yellow photoderivative [20% yield on the starting material after crystallization from $CHCl_3$–CH_3OH; it blackens without melting over 250°; $\lambda_{max}^{CHCl_3}$ = 446 nm (ε 58,800); ν_{max} = 3420, 3260, 1695, 1645, 1615 cm^{-1} (in $CHCl_3$)] to which the formula $C_{34}H_{40}N_4O_7$ could be attributed on the basis of its mass-spectrum [*m/e* 616 (2.5%, M^+), 584 (37%), 286 (100%)] and its elemental analysis[6]. In addition, this photopigment gave a positive diazoreaction[7] and afforded bilirubin when its chloroform solution was stirred with aqueous HCl 10*N* for 2 h at r.t. In the NMR-spectrum, as shown in the Figure, it exhibited the characteristic ABC pattern of 1 vinyl group[8], which could be analyzed as an ABX system[9] (6 lines in the X part and 8 lines in the AB part: δ_A, δ_B, δ_X = 5.53, 5.38, 6.59 ppm; J_{AX}, J_{BX}, J_{AB} = 17.2, 10.3, 1.7 Hz), and further a set of peaks associated to the grouping $-CH(OCH_3)-CH_3$ [a singlet at δ 3.21 (3H, $-OCH_3$), a doublet centered at δ 1.40 (3H, J = 7 Hz, $-CH_3$) and a quartet centered at δ 4.32 (1H, J = 7 Hz,



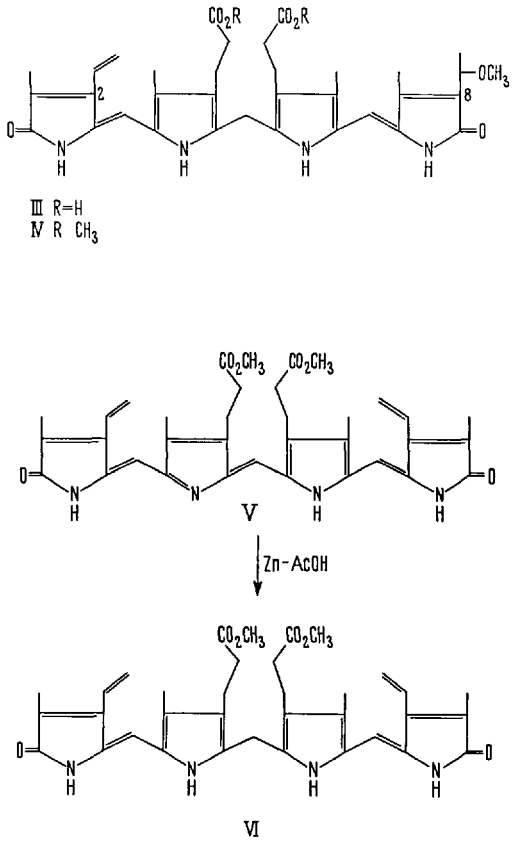
I R=H
II R=CH_3



NMR-spectrum at 60 MHz of 8a-methoxy-8a,8b-dihydrobilirubin (III) in $CDCl_3$. Chemical shifts are in ppm (δ) from internal tetramethylsilane.

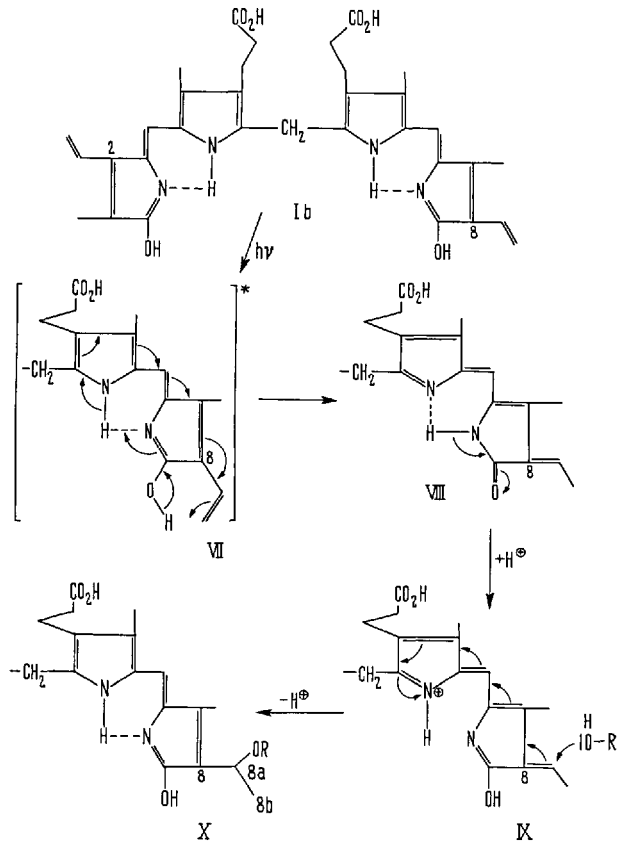
$\geqslant$CH); interrelation between the last 2 groups of signal being provided by a spin decoupling experiment].

All these data suggested that the photopigment arose from Markownikoff addition of a molecule of methanol to 1 vinyl double bond of bilirubin, but did not distinguish between the 2 possible isomers resulting from such a reaction. The unambiguous assignement of the structure III to the photoadduct rests on spectroscopic evidence, as follows.



Treatment of III with the stoichiometric amount of diazomethane in $CHCl_3$ for 1 min, followed by precipitation with light petroleum, afforded the corresponding dimethyl ester (IV) [ν_{max} = 3315, 1740 cm^{-1} (in $CHCl_3$); *m/e* 644 (M^+), 612] the NMR-spectrum of which was compared with that of bilirubin dimethyl ester (II) prepared in the same manner[10]. From such a comparison[11] it was found that the downfield vinyl group of bilirubin dimethyl ester (ABX system showing δ_A, δ_B, δ_X = 5.40, 5.24, 6.55 ppm and J_{AX}, J_{BX}, J_{AB} = 17.5, 12.1, 1.4 Hz) was again present in the photoderivative dimethyl ester (ABX system showing δ_A, δ_B, δ_X = 5.42, 5.30, 6.53 ppm and J_{AX}, J_{BX}, J_{AB} = 17.5, 12.1, 1.4 Hz). In order to establish whether this vinyl group was in position 2 (*endo*) or 8 (*exo*) of the biladiene-*a,c* skeleton, we synthesized the symmetrical compound VI containing both vinyl substituents in *endo*-β-position of the outer pyrrole rings. This isomeric bilirubin dimethyl ester (VI) [$\lambda_{max}^{CHCl_3}$ = 405 nm (ε 63,000); *m/e* 612 (4.5%, M^+), 300 (100%)] was obtained in moderate yield by treating the corresponding verdin V[12] with Zn in acetic acid under nitrogen[13] and purifying the crude product of reduction by preparative TLC (Merck Silica Gel G, 1.5 mm, 10% ethanol in benzene).

In the NMR-spectrum of VI, the signals due to the vinyl protons (ABX system showing δ_A, δ_B, δ_X = 5.46, 5.33, 6.57 ppm and J_{AX}, J_{BX}, J_{AB} = 17.5, 12.0, 1.4 Hz) appeared to fall into the same range as the downfield vinyl group of bilirubin dimethyl ester (II). This finding, which indicated that the downfield vinyl of II was to be placed in position 2 (*endo*), allowed the structure III to be assigned to the product formed by photoaddition of methanol to bilirubin.



[1] J. LUCEY, M. FERREIRO and J. HEWITT, Pediatrics *41*, 1047 (1968) and references cited therein.
[2] J. D. OSTROW and R. V. BRANHAM, Gastroenterology *58*, 15 (1970).
[3] Bearing in mind the well recognized possibility of tautomerism between a lactam and enol form in the outer pyrrole rings of rubins and verdins[7], no attempt will be made to represent the actual structures for the compounds mentioned here, except where it will be pertinent to the discussion.
[4] Irradiations were conducted under nitrogen using a Philips HPK 125 W high-pressure mercury lamp at ca. 40 °C.
[5] Z. J. PETRYKA and C. J. WATSON, J. Chromat. *37*, 76 (1968).
[6] Satisfactory analyses, IR-, NMR- and MS-spectra were recorded for all the compounds described.
[7] T. K. WITH, *Bile Pigments* (Academic Press Inc., New York and London 1968).
[8] E. WENKERT and P. BEAK, J. Am. chem. Soc. *83*, 998 (1961).
[9] J. W. EMSELEY, J. FEENEY and L. H. SUTCLIFFE, *High Resolution Nuclear Magnetic Resonance Spectroscopy* (Pergamon Press, London 1965), vol. 1, p. 357. – T. SCHAEFER, Can. J. Chem. *40*, 1 (1962).
[10] H. FISCHER and H. ORTH, *Die Chemie des Pyrrols* (Akad. Verlagsgesellschaft, Leipzig 1937), Bd. 2, Tl. 1, p. 637.
[11] Comparison between the NMR-spectra of bilirubin and its photoderivative was not possible because the solubility of the former is too low in chloroform to obtain a useful spectrum.
[12] This compound was prepared according to R. BONNET and A. F. MCDONAGH, Chem. Commun. *1970*, 238.
[13] A. W. NICHOL and D. B. MORELL, Biochim. biophys. Acta *184*, 173 (1969).

We found that, besides methanol, other alcohols can be added photochemically to bilirubin giving 8a-alkoxy-8a,8b-dihydrobilirubins (X). Thus, 8a-ethoxy- (X, R = C_2H_5), 8a-*n*-propoxy- (X, R = $CH_2CH_2CH_3$) and 8a-*iso*-propoxy-8a,8b-dihydrobilirubin (X, R = $CH(CH_3)_2$) were obtained[6] when bilirubin was irradiated in chloroform containing ethanol, *n*-propanol and *iso*-propanol, respectively. It is noteworthy that these products, as well as III, were found to be the only ones formed in significant yield under the above conditions. The mechanism suggested to account for the formation of all the photoadducts is outlined below.

The finding that photoinduced addition of protic reagents (ROH) to bilirubin occurs preferentially at the *exo*-vinyl group and follows Markownikoff's rule, may be rationalized by assuming the attack by the nucleophile at position 8a of a cation such as IX. The formation of IX is best explained as involving a protonation of the basic intermediate VIII arising from a rearrangement of an electronically excited state of bilirubin (VII), although an external protonation of the same excited state to form IX directly, cannot be ruled out a priori. The assumption of the rearrangement outlined in VII is consistent with the finding that bilirubin exists in the enol form (Ib)[14]; furthermore, according to the process shown in the scheme, formation of III was observed when methanol was added to a previously irradiated chloroform solution of bilirubin[15].

The reaction which results from irradiation of bilirubin in the presence of a nucleophile is a unique example of a photochemically induced ionic addition to a vinyl double bond[16] and raises the possibility that at least part of the serum bilirubin in new-born infants may be eliminated during phototherapy as photoadducts with nucleophilic substances occurring in the body.

Riassunto. Per azione della luce ultravioletta la bilirubina disciolta in $CHCl_3$–CH_3OH somma una molecola di metanolo fornendo un prodotto per il quale si dimostra la struttura III. Tale reazione, osservata anche con altri alcooli, suggerisce la possibilità che parte della bilirubina del siero dei neonati itterici sottoposti a fototerapia venga eliminata sotto forma di prodotti di fotoaddizione con sostanze nucleofile presenti nell'organismo.

P. MANITTO[17]

Istituto di Chimica Organica della Facoltà di Scienze dell'Università di Milano, Centro per lo studio delle sostanze organiche naturali del C.N.R., Via Saldini 50, I-20133 Milano (Italy), 9 March 1971.

[14] A. W. NICHOL and D. B. MORELL, Biochim. biophys. Acta *177*, 599 (1969); it is likely that the photoadducts we report here are also in the enol form in chloroform solution.

[15] Eventually, the above mechanism is supported by the observed pH dependence of photoadduct formation (faster at lower pH) under irradiation; pH of the medium was varied by adding acetic acid or triethylamine.

[16] J. A. MARSHALL, Acct chem. Res. *2*, 33 (1969).

[17] The author wishes to thank Prof. L. CANONICA for helpful discussions, Dr. G. SEVERINI RICCA for running the NMR-spectra and Dr. T. SALVATORI for the mass-spectra.

Teratologische Prüfung einiger Thalidomid-Metabolite

Thalidomid bildet im Organismus durch Hydrolyse zahlreiche Metabolite[1]. Zur Klärung der Frage, ob Thalidomid selbst oder eines der Hydrolysenprodukte das eigentliche teratogene Agens darstellt, wurden die aufgefundenen Spaltprodukte synthetisiert und am Kaninchen teratologisch geprüft[2]. In keinem Falle liess sich eine eindeutige teratogene Wirkung beobachten. Dieser Befund könnte nach KEBERLE[3] darauf zurückgehen, dass für die Metabolite auf Grund ihrer polaren Struktur Membranschranken bestehen, während für Thalidomid weder das Endometrium und die Blastocystenmembranen noch die Placenta Hindernisse darstellen.

Um eine höhere Membrangängigkeit der Metabolite zu erreichen[4], lösten wir die zu prüfenden Substanzen mit Hilfe des Tensides Tween 20 und applizierten die relativ hochkonzentrierten Lösungen intraperitoneal. Unter diesen Bedingungen zeigte N-Phthalyl-DL-glutaminsäure (I) embryotoxische Effekte bei der SWS-Maus[5]. Ferner wurde nachgewiesen, dass die biologische Aktivität von I ausschliesslich auf das L-Isomere zurückgeht[6]. Die embryotoxische Wirkung des L-Isomeren konnte durch konkurrierende Verabreichung von L-Glutaminsäure, nicht aber durch D-Glutaminsäure kompensiert werden[7].

In der vorliegenden Arbeit werden die Hydrolysenprodukte von I teratologisch untersucht: Die durch Aufspaltung des Phthalimid-Ringes entstehende N-(*o*-Carboxybenzoyl)-DL-glutaminsäure (II) sowie die aus II nach einem weiteren hydrolytischen Schritt resultierenden Spaltprodukte Phthalsäure (III) und Glutaminsäure (IV). Die Substanzen II und III wurden nach Gabe von Thalidomid in Urin und Faeces nachgewiesen[1,3]; IV fand sich nach Hydrolyse von Thalidomid unter physiologischen Bedingungen[8].

Zur Herstellung von II werden 2,77 g (10 m*M*) I[9] unter Eiskühlung und Schütteln mit 15,3 ml 2*N* NaOH versetzt. Man gibt die Lösung sogleich auf eine 2 × 13 cm-Säule von Dowex 50 ($H^{\oplus}$-Form) und fängt etwa 60 ml Eluat (pH ≦ 3) in einer eisgekühlten Vorlage auf. Dabei kristallisiert chromatographisch reines II aus. Das Produkt wird