





FIGURE II SHIFT DIAGRAMS FOR AZOBILIRUBIN AND AZOMESOBILIRUBIN ISOMERS

azo pigments, we considered that a carbon-13 nmr study could provide considerable structural information of unknown bilirubin compounds (8).

The present communication is concerned with the assignments of the carbon-13 chemical shifts of azobilirubin (II-III) and azomesobilirubin (IV-V) isomers (9,10).

Carbon-13 chemical shifts for the peripheral substituents on rings A and B in the series of compounds studied are given in Table I. Spectral assignments were made essentially on the basis of comparing chemical shifts of the collapsed multiplets induced by total proton decoupling. The resonances of the substituents on ring A in the four isomers shown in Figure I can readily be identified by comparison with substituted pyrroles and porphyrins (11). The alkyl and the azophenyl groups attached to ring A remain without significant change in the four azo pigments. The *ipso*-carbon  $C_1$  of the phenylazo group appears at low field with shifts resembling the carbon atoms of phenylhydrazine, azobenzene or *N*-benzylideneanilines (12). The remaining benzene carbon atoms are easily ascribed from intensity and chemical shift considerations. The methine bridge carbon signals are found at high field in the spectra of mesobilirubin derivatives, compared with those compounds having vinyl group substituents. The same behavior is observed in most of the porphyrins where the methine bridge is binding two pyrrole moieties. The introduction of a vinyl or methyl ketone substituent on the same side of the methine group generally results in the deshielding of the methine carbon atom (13).

Figure II is a graphic comparison of the corresponding shifts of the saturated and unsaturated vinyl

group isomers. The  $sp^2$  carbon atoms exhibit absorption between 115-175 ppm with small variations existing between compounds. The position of carbon  $C_2$  on the pyrrole ring is affected when the vinyl group is hydrogenated. The paramagnetic shift magnitude for this carbon atom is  $\sim 1.7$  ppm in the couple

Table I

Chemical Shifts of the Substituents of Bilirubin Derivatives (a)  
(In Ppm from Internal TMS)

	II	III	IV	V
Methine	97.8	98.1	96.7	96.6
Py <sub>A</sub> -CH <sub>2</sub> (t)	20.2	19.8	20.0	20.0
CH <sub>2</sub> (t)	34.8	34.7	34.8	34.8
C=O (s)	173.6	173.3	173.6	173.6
OCH <sub>3</sub> (q)	51.7	51.4	51.7	51.6
Py <sub>A</sub> -CH <sub>3</sub> (q)	9.6	9.6	9.6	9.6
Py <sub>B</sub> -CH (d)	125.8	128.7	---	---
CH <sub>2</sub> (t)	121.7	122.6	---	---
Py <sub>B</sub> -CH <sub>3</sub> (q)	9.6	9.4	9.4	8.5
Py <sub>B</sub> -CH <sub>2</sub> (t)	---	---	17.0	17.5
CH <sub>3</sub> (q)	---	---	13.2	14.0
N=N-C <sub>i</sub> (s)	153.1	153.1	153.4	153.4
C <sub>o</sub> (d)	116.0	115.5	115.7	115.6
C <sub>m</sub> (d)	129.4	129.1	129.4	129.4
C <sub>p</sub> (d)	124.1	123.4	123.6	123.6

(a) Py<sub>A</sub> = pyrrole ring; Py<sub>B</sub> = pyrrolinone ring.

II-IV and  $\sim 1.2$  ppm in the III-V pair as shown in Figure IIab. Since carbon C<sub>2</sub> is bound to two nitrogen nuclei, its signal appears as a broad singlet in a region relatively free of other signals.

The carbon atoms corresponding to the vinyl groups were readily identified from the longer chemical shift changes induced by hydrogenation. Furthermore, hydrogenation also causes chemical shift changes in the carbon atoms of ring B in both the azobilirubin and azomesobilirubin pair of isomers. The  $\beta$ -substituents to the carbonyl group are shifted more than those attached to the  $\alpha$ -position. A greater anisotropic effect is observed in the pyrrolinone carbonyl signal when the vinyl or ethyl group is bonded on the  $\beta$ -carbon C<sub>3'</sub>. The small variations induced in the C<sub>5'</sub> signals allow distinction between the carbon signals arising from the side chain carbonyl group.

The ester carbonyl carbon positions are virtually the same, showing absorption between 173.3 to 173.6 ppm as shown in Table I. The frequencies of the signals resulting from the lactam and ester carbon atoms correspond adequately with the assignments previously made in earlier carbon-13 nmr studies (14). In pair II and IV shown in Figure IIa, carbon C<sub>4'</sub> is shifted by  $\delta$  7.7 ppm; the other carbon atoms C<sub>3'</sub> and C<sub>2'</sub> remain almost unaffected. However, carbons C<sub>3'</sub> and C<sub>2'</sub> in compounds III and V shown in Figure IIb are shifted by  $\delta$  4.9 and  $\delta$  3.4 ppm, respectively. The change for carbon C<sub>2'</sub> is rationalized in terms of lost cross conjugation. The chemical shifts of the nitrogen containing rings are given directly on the corresponding formulae and can be seen in the bar diagram in Figure II. The foregoing results could be important for current and future research on bilirubin metabolites, bilirubin isomers and photodegradation products of bilirubin.

#### EXPERIMENTAL

Compounds II-IV were prepared as described previously (9,10). The carbon-13 nmr spectra were determined in the pulse FT mode using deuteriochloroform solutions on a Bruker at 22.63 MHz and in a Varian Associates CFT-20 spectrometer. The chemical shifts were extracted from proton noise decoupled spectra and the number of directly attached hydrogen atoms was established from off resonance cw decoupling experiments.

Acknowledgement.

We thank Professor J. M. Muchowski of Syntex and Dr. K.

Jankowski of the University of Moncton for their help in this study.

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