Kurze Mitteilungen

Bis am 15. des Monats bei der Redaktion eingehende Kurze Mitteilungen werden in der Regel am 15. des folgenden Monats veröffentlicht. Es werden auch Manuskripte aus dem Ausland angenommen

Carbohydrates of Bovine α-Lactalbumin Preparations*

Summary

D-mannose, D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose and N-acetyl neuraminic acid have been identified in preparations of bovine α-lactalbumin. The preparations were fractionated by ion-exchange chromatography in an attempt to determine the origin of the carbohydrates. The existence of two minor components associated with α-lactalbumin, and differing markedly in their carbohydrate composition, is suggested. One component contained most of the carbohydrates in the preparations, and appeared similar to that isolated by BARMAN[1] whilst the other minor component contained principally hexosamine, and appeared similar to that isolated by GORDON *et al.*[2]

Introduction

The presence of hexosamine on a minor component of α-lactalbumin was reported by GORDON *et al.*[2] and since the completion of the present work, BARMAN[1] has identified mannose, fucose, galactose, galactosamine, glucosamine and N-acetyl neuraminic acid on a minor component of α-lactalbumin isolated by ion-exchange chromatography. In this investigation we have fractionated α-lactalbumin preparations by ion-exchange chromatography in an attempt to determine the origin of the carbohydrates in the preparation. A brief description of this work has been reported previously[3].

Experimental

All milk samples were from different individual cows which were known to be free of bacterial infections of the udder. α-lactalbumin was prepared by the method of ASCHAFFENBURG[4] and by ammonium sulphate fractionation followed by separation on Sephadex G-100 (Pharmacia, Uppsala, Sweden). The α-lactalbumin preparations were purified by ion-exchange chromatography on diethylaminoethyl cellulose, DE-52 (W. and R. BALSTON, Maidstone) using the method of BRODBECK *et al.*[5]

All samples were dialysed for 5 days at 4° against tap-water which was changed twice daily, to remove any small molecules, including free carbohydrates. The samples were the evaporated to dryness and stored over P_2O_5 for 12 h. Carbohydrate analysis was performed by gas-liquid chromatography of the trimethyl silyl derivatives of the methyl glycosides[6,7] released on methanolysis (4 h at 80° in 0.64 M HCl in anhydrous methanol). Carbohydrate analysis prior to methanolysis showed that all the samples were free of unbound carbohydrates.

Polyacrylamide gel disc electrophoresis (tris/glycine, *p*H 8.1) was performed on all the α-lactalbumin preparations using a method based on those of ORNSTEIN[8] and DAVIES[9].

Results

The carbohydrate analysis showed that D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose were present in all samples of α-lactalbumin (Table I). N-acetyl neuraminic acid was detected in relatively small amounts in only 3 of the 5 samples analysed. On polyacrylamide gel disc electrophoresis, all preparations showed one major band and a faster moving minor band. No other bands were detected in samples 4 and 5, but in samples 1 and 2, a slower moving band was also detected.

The ion-exchange chromatography elution profiles (Fig. 1) show that all the preparations contained one major peak with minor peaks being eluted immediately before and after this major peak. It was established by gel electrophoresis that the main peak (fraction C) was α-lactalbumin, fraction B contained the slower moving band, fraction D contained both the main peak and the faster moving component, and fraction E contained only the faster moving minor band.

Carbohydrate analysis of the individual fractions (Table II) showed that there were comparatively high values for D-galactose, 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose in fraction D and, in some samples, in fraction E. The values for the carbohydrates in fraction B were high, whilst those in the main α-lactalbumin fraction, C, contained low concentrations of carbohydrates.

Discussion

Our experiments confirm that there are carbohydrates present in preparations of α-lactalbumin and suggest that the carbohydrates are present in minor components associated with the main α-lactalbumin molecule, which appears to be devoid of carbohydrates. These results are in general agreement with those of GORDON *et al.*[2] and of BARMAN[1]. There was quite a variation between samples in the concentrations of carbohydrates but this is in

* Received January 1, 1971.

1 T.E. BARMAN, *Biochim. Biophys. Acta 214* (1970) 242.
2 W.G. GORDON, R. ASCHAFFENBURG, A. SEN and S.K. GHOSH, *J. Dairy Sci. 51* (1968) 947.
3 E.J. HINDLE and J.V. WHEELOCK, *Biochem. J. 119* (1970) 14P.
4 R. ASCHAFFENBURG, *J. Dairy Sci. 51* (1968) 1295.
5 U. BRODBECK, W.L. DENTON, N. TANAHASHI and K.E. EBNER, *J. Biol. Chem. 242* (1967) 1391.
6 J.R. CLAMP, G. DAWSON and L. HOUGH, *Biochim. Biophys. Acta 148* (1967) 342.
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8 L. ORNSTEIN, *Ann. N. Y. Acad. Sci. 121* (1964) 321.
9 B.J. DAVIES, *Ann. N. Y. Acad. Sci. 121* (1964) 404.

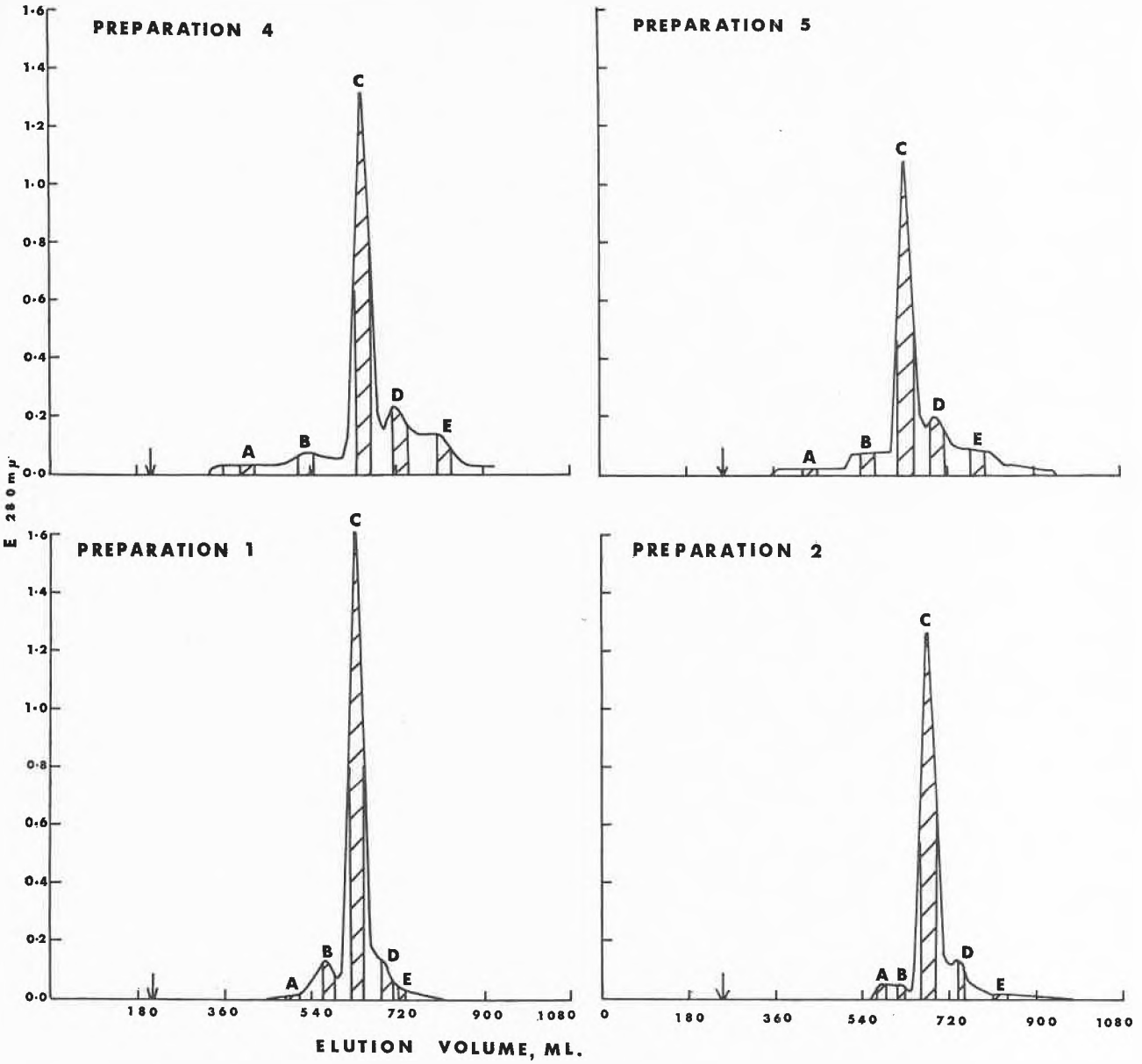

Fig. I. Ion-exchange chromatography of α-lactalbumin preparations. Details of the preparations are as shown in Table I. The proteins were eluted with a gradient of 20–250 mM tris-HCl containing 5 mM $MgCl_2$ adjusted to *p*H 7.8. This was obtained by using 2 flasks each containing 500 ml buffer. The concentration of tris was 20 mM in the first flask and 388 mM in the second. Chromatography was performed at 4° and the fractions were monitored at 280 mμ against a buffer blank. The arrow marks the point at which the gradient was started. Those fractions which were analysed for carbohydrates are indicated by the shaded areas

Table I. Concentrations of carbohydrates in the α-lactalbumin preparations

α-lactalbumin preparation	D-mannose %	D-galactose %	2-acetamido-2-deoxy-D-galactose %	2-acetamido-2-deoxy-D-glucose %	N-acetyl neuraminic acid %
1	0.86	0.29	0.51	0.29	0.09
2	0.49	0.18	0.04	0.05	0.03
3	0.06	0.11	0.22	0.11	0.00
4	0.36	0.05	0.07	0.09	0.00
5	0.50	0.14	0.20	0.35	0.06

Samples 1 and 2 were prepared by the method of ASCHAFFENBURG[4].
Sample 3 was prepared by ammonium sulphate fractionation of whole milk followed by separation on Sephadex G-100.
Sample 4 was supplied by Nutritional Biochemicals Corporation, Cleveland (Ohio).
Sample 5 was supplied by Koch-Light Laboratories Ltd., Colnbrook (Bucks.).

Table II. Concentrations of carbohydrates in the fractions isolated by ion-exchange chromatography and which contain α-lactalbumin. Details of the preparations are given in Table I

α-lactalbumin preparation	fraction	D-mannose	D-galactose	2-acetamido-2-deoxy-D-galactose	2-acetamido-2-deoxy-D-glucose	N-acetyl neuraminic acid
		expressed as a % of α-lactalbumin				
1	B	1.60	1.12	1.38	4.40	0.36
	C	0.04	0.07	0.02	0.04	0.00
	D	0.15	0.42	0.23	0.24	0.10
	E	0.55	0.86	0.42	0.38	0.00
2	B	3.06	1.31	1.98	1.13	0.00
	C	0.04	0.04	0.06	0.07	0.00
	D	0.11	0.72	0.45	0.51	0.00
	E	0.19	0.61	1.55	1.32	0.00
4	B	0.14	0.07	0.13	0.44	0.00
	C	0.08	0.11	0.09	0.05	0.00
	D	0.16	0.50	0.45	0.32	0.00
	E	0.23	0.63	1.50	1.03	0.00
5	B	1.49	0.58	0.42	0.73	0.00
	C	0.07	0.05	0.05	0.07	0.00
	D	0.21	0.74	0.29	0.38	0.00
	E	0.14	0.31	0.32	0.17	0.00

The values have been calculated on the assumption that the protein is α-lactalbumin.

general agreement with information obtained for various other glycoproteins.

A number of investigations have shown that there is a minor component associated with α-lactalbumin which moves faster than the main component on electrophoresis at a range of *p*H values (WETLAUFER[10], by paper electrophoresis, *p*H 7.5; ASCHAFFENBURG and DREWRY[11], by paper electrophoresis, *p*H 8.6; GORDON *et al.*[2], by paper electrophoresis, *p*H not stated; KRONMAN and VITOLS[12], by starch gel electrophoresis, *p*H 8.55). This is probably the same minor component which we have identified in fractions D and E obtained by ion-exchange chromatography.

On the other hand, the component in fraction B, which was eluted prior to the main α-lactalbumin peak on ion-exchange chromatography (*p*H 7.8) moved more slowly than the main component on gel electrophoresis (*p*H 8.1). Recently, BARMAN[1] has isolated an α-lactalbumin component which showed similar characteristics on ion-exchange chromatography (*p*H 8.5) and on gel electrophoresis (*p*H 8.6). This behaviour under approximately similar conditions differs from the minor components isolated by GORDON *et al.*[2] and so it is unlikely to be the same as that isolated by the other workers. In fact, there was a faster-moving component on the gel electrophoresis of BARMAN's α-lactalbumin preparation. This was not eluted from the ion-exchange column possibly because the concentration of the buffer was too low (i.e. 100 mM tris). In our experiments the concentration of the buffer was approximately 250 mM tris when the minor component in fraction D was eluted. This evidence suggests that the minor component which we isolated in fraction B is probably the same as that studied by BARMAN[1] and that the faster-moving component, which he observed on gel electrophoresis is the same as that isolated by us in fractions D and E.

The results for the concentrations of carbohydrates present in fraction B are similar to those of BARMAN[1] with the exception that fucose was not identified. The concentration of carbohydrates in this fraction, however, are markedly higher than those obtained from the minor component eluted in fractions D and E. This minor component has relatively higher concentrations of 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose and D-galactose, which is in agreement with GORDON *et al.*[2], who reported the presence of 1% hexosamine on a minor component of α-lactalbumin. The minor component isolated by BARMAN, however, contained approximately 5% hexosamine, which is further evidence for the difference between the minor components of BARMAN[1] and of GORDON *et al.*[2]

In conclusion, we believe that there may be at least 2 minor components accompanying α-lactalbumin which contain carbohydrates, but which differ in their carbohydrate composition.

Acknowledgements

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[10] D. B. WETLAUFER, *C. R. Trav. Lab. Carlsberg 32* (1961) 125.
[11] R. ASCHAFFENBURG and J. DREWRY, *Biochem. J. 65* (1957) 273.
[12] M. J. KRONMAN and R. VITOLS, unpublished data cited by M. J. KRONMAN and R. E. ANDREOTTI, *Biochemistry 3* (1964) 1145.

*Sur la possibilité de relier le spectre de masse à la fragmentation radiolytique par le calcul matriciel**

Summary

A new semi-empirical relation is proposed to relate the radiolytic ionic fragmentation in the vapor phase to the mass spectrum of hydrocarbons. Extensive use is made of the cross sections of the various ion-molecule reactions of the fragment ions. Numerical calculation are carried out for cyclohexane.

Le présent travail se propose d'examiner un modèle semi-empirique de liaison entre la fragmentation observée dans les hydrocarbures en spectrométrie de masse et en radiolyse en phase vapeur. Admettons que la fragmentation initiale soit identique en spectrométrie de masse à haute énergie (70 eV) et en radiolyse, comme l'a suggéré PLATZMAN[1], et comme l'ont démontré LIAS et AUSLOOS dans le cas particulier de la formation de $C_4H_8^+$ à partir de *c*-$C_6H_{12}^+$[2]. Dans un tel cas le nombre de ions formés en radiolyse sera supposé proportionnel à l'intensité I du pic correspondant dans le spectre de masse.

Tout ion de fragmentation réagit avec la molécule mère selon des réactions ion-molécule. Dans le cas du cyclohexane, les réactions de ses ions de fragmentation ont été déterminées par ABRAMSON et FUTRELL[3] au spectromètre de masse tandem : à basse énergie (0,4 eV), elles se limitent aux quatre réactions (1) à (4) :

$$C_nH_m^+ + c\text{-}C_6H_{12} \longrightarrow \begin{cases} C_nH_m + C_6H_{12}^+ & (1) \\ C_nH_{m+1}^{\cdot} + C_6H_{11}^+ & (2) \\ C_nH_{m+2} + C_6H_{10}^+ & (3) \\ C_nH_{m+1}^+ + C_6H_{11}^{\cdot} & (4) \end{cases}$$

La réaction (4) ne se produit qu'avec les ions $C_4H_8^+$ et avec une probabilité relative de l'ordre de 10%. Elle sera négligée dans la suite de ce travail.

L'absence de réactions de condensation ionique rend ce système particulièrement simple à étudier, car chaque fragment neutre d'origine ionique ne possède au maximum que trois précurseurs différents. Le rendement radiolytique G de chaque fragment neutre formé selon (1) à (3) sera la somme de trois termes :

$$G(C_nH_i) = k_1[C_nH_i^+] + k_2[C_nH_{i-1}^+] + k_3[C_nH_{i-2}^+]. \quad (5)$$

Il existe autant de relations (5) que de produits C_nH_i formés en radiolyse. L'ensemble de toutes ces relations peut être condensé à l'aide d'une représentation matricielle.

Considérons tout d'abord l'ensemble des ions, radicaux, et molécules ayant le même nombre n d'atomes C. On peut définir un vecteur $\tilde{I}_n$ dont les composantes I_{ni} sont les intensités relatives des pics $C_nH_i^+$ du spectre de masse, et un vecteur $\tilde{G}_n$ dont les composantes G_{nj} sont les rendements radiolytiques G des fragments C_nH_j, où i et j varient tous deux de 0 à $2n + 2$. Si j est impair, C_nH_j est un radical dont on suppose qu'il se recombine intégralement au radical cyclohexyle, et dont le G se mesure par celui du cyclohexane substitué correspondant. Ce formalisme ne distingue pas les isomères : $G(C_3H_7)$ comprend donc la somme des propyl- et isopropylcyclohexane, et donne la composante G_{37}. Si les fragments C_nH_j de radiolyse sont formés selon (1) à (3), les deux vecteurs $\tilde{G}_n$ et $\tilde{I}_n$ sont reliés l'un à l'autre par une matrice carrée $\tilde{S}_n$ selon (6) dont les éléments S_{nij} sont proportionnels à la section efficace ou à la constante de vitesse de la réaction transformant le ion $C_nH_i^+$ en fragment neutre C_nH_j.

$$\tilde{G}_n = \alpha \tilde{S}_n \tilde{I}_n \quad (6)$$

où α est un facteur de proportionnalité indépendant de n, i et j. La matrice $\tilde{S}_n$ possède des éléments S_{nji} qui sont tous nuls sauf les éléments de la diagonale principale S_{nii} et les deux éléments immédiatement inférieurs $S_{n,i+1,i}$ et $S_{n,i+2,i}$.

On évite la nécessité d'introduire le facteur α en remplaçant $\tilde{G}_n$, $\tilde{I}_n$ et $\tilde{S}_n$ par leurs valeurs relatives $\tilde{G}'_n$, $\tilde{I}'_n$ et $\tilde{S}'_n$ tels que

$$G'_{nj} = G_{nj}/\sum_j G_{nj}, \quad I'_{ni} = I_{ni}/\sum_i I_{ni}, \quad S'_{nji} = S_{nji}/\sum_i S_{nji}.$$

Dans ce cas les vecteurs $\tilde{G}'$ et $\tilde{I}'$ sont reliés par la relation

$$\tilde{G}'_n = \tilde{S}'_n \tilde{I}'_n.$$

L'applicabilité d'un tel formalisme peut être testée dans le cas de la fragmentation ionique du cyclohexane dont les sections efficaces des réactions (1) à (4) ont été déterminées[3]. Ce modèle ne peut être appliqué qu'aux fragments radiolytiques d'origine ionique formés par (1) à (3). Il ne peut donc décrire la formation des fragments en C_1 et C_2, qui sont issus de la fragmentation initiale du ion moléculaire et non des réactions (1) à (3).

Avec $n = 3$, si l'on suppose que tous les produits de radiolyse sont d'origine ionique, on a :

$$\tilde{G}_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0{,}03 \\ 0{,}04 \\ 0{,}70 \\ 0{,}12 \\ 0{,}31 \end{bmatrix} \quad \tilde{G}'_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0{,}03 \\ 0{,}04 \\ 0{,}59 \\ 0{,}10 \\ 0{,}25 \end{bmatrix} \quad \tilde{I}_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 238 \\ 11 \\ 620 \\ 239 \\ 132 \\ 0 \end{bmatrix} \quad \tilde{I}'_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0{,}187 \\ 0{,}010 \\ 0{,}490 \\ 0{,}228 \\ 0{,}104 \\ 0 \end{bmatrix}$$

* Présenté à l'assemblée de printemps de la Société Suisse de Chimie, 7 mai 1971 à Lausanne.

[1] R. L. PLATZMAN, *Radiation Res. 17* (1962) 419.

[2] S. G. LIAS et P. AUSLOOS, *J. Amer. Chem. Soc. 92* (1970) 1840.

[3] F. P. ABRAMSON et J. H. FUTRELL, *J. Physic. Chem. 71* (1967) 3791.

[4] M. COSANDEY, *Helv. Chim. Acta*, à l'impression.

Les valeurs numériques des G proviennent d'une publication antérieure[4], celles des I sont tirées de la compilation de CORNU et MASSOT[5]. Les éléments de $\widetilde{S}_3$ ne sont pas tous connus. Les plus importants ont été déterminés par ABRAMSON et FUTRELL[3] et figurent dans le tableau suivant ou ils sont indiqués en Å[2] pour des ions de 0,3 eV:

$$\widetilde{S}_3 = \begin{array}{c} i=4 \quad 5 \quad 6 \quad 7 \quad 8 \quad j \\ \begin{pmatrix} \ldots & 0 & 0 & 0 & 0 \\ \ldots & 60 & 9 & 0 & 0 \\ \ldots & 0 & 39 & 0 & 0 \\ \ldots & 0 & 26 & 37 & 0 \end{pmatrix} \begin{array}{c} 5 \\ 6 \\ 7 \\ 8 \end{array} \end{array}$$

donc:

$$\widetilde{S}'_3 = \begin{array}{c} \\ \begin{pmatrix} \ldots & 0 & 0 & 0 & 0 \\ \ldots & 1 & 0{,}12 & 0 & 0 \\ \ldots & 0 & 0{,}53 & 0 & 0 \\ \ldots & 0 & 0{,}35 & 1 & 0 \end{pmatrix} \begin{array}{c} j \\ 5 \\ 6 \\ 7 \\ 8 \end{array} \end{array}$$

$$\widetilde{S}'_3 \cdot \widetilde{I}'_3 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ \text{n.d.} \\ \text{n.d.} \\ 0{,}53 \\ 0{,}13 \\ 0{,}18 \end{bmatrix}$$

n.d. signifie «non déterminé»

On constate que malgré la grossièreté du modèle, l'accord entre la valeur $\widetilde{G}'_3$ et $\widetilde{S}'_3\widetilde{I}'_3$ est satisfaisant en première approximation. Pour $n = 4$ on a de même,

$$G_4 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0{,}45 \\ 0{,}10 \\ 0{,}32 \end{bmatrix} \quad \widetilde{G}'_4 - \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0{,}52 \\ 0{,}11 \\ 0{,}37 \end{bmatrix} \quad \widetilde{I}_4 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 340 \\ 1000 \\ 0 \\ 0 \end{bmatrix} \quad \widetilde{I}'_4 = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0{,}25 \\ 0{,}75 \\ 0 \\ 0 \end{bmatrix}$$

Seuls les éléments de matrice pour lesquels i et j sont $\geqslant 7$ sont connus, mais ils suffisent pour le cas présent.

[5] A. CORNU et R. MASSOT, *Compilation of Mass Spectral Data*, Heyden & Sons, London 1966.

$$\widetilde{S}'_4 = \begin{array}{c} i=7 \quad 8 \quad 9 \quad 10 \quad j \\ \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0{,}14 & 0 & 0 \\ 0 & 0{,}85 & \text{n.d.} & 0 \end{pmatrix} \begin{array}{c} 8 \\ 9 \\ 10 \end{array} \end{array}$$

D'où:

$$\widetilde{S}'_4 \cdot \widetilde{I}'_4 - \begin{pmatrix} \ldots \\ 0 \\ 0 \\ 0{,}25 \\ 0{,}10 \\ 0{,}64 \end{pmatrix} \begin{array}{c} 6 \\ 7 \\ 8 \\ 9 \\ 10 \end{array}$$

L'écart entre $\widetilde{G}'_4$ et $\widetilde{S}'_4\widetilde{I}'_4$ provient du fait que le calcul de $\widetilde{S}'_4 \cdot \widetilde{I}'_4$ ne distingue pas entre les deux ions 1-$C_4H_8^+$ et 2-$C_4H_8^+$ dont les abondances et les constantes de vitesse sont très différentes. En effet, globalement pour l'ensemble des réactions (1) à (4), les ions 1-$C_4H_8^+$ sont 62 fois plus réactifs[3] que les ions 2-$C_4H_8^+$. Si l'on admet avec ABRAMSON et FUTRELL[3] qu'en radiolyse et en spéctrométrie de masse, 80% des ions $C_4H_8^+$ sont sous forme 2-$C_4H_8^+$, et que la moitié de ces ions se neutralisent purement et simplement[4], on obtient alors

$$\widetilde{S}'_4 \cdot \widetilde{I}'_4 = \begin{array}{c} \\ \begin{pmatrix} 1 \cdot 0{,}25 + 0{,}5 \cdot 0{,}8 \cdot 0{,}75 \\ 0{,}2 \cdot 0{,}14 \cdot 0{,}75 + 0{,}14 \cdot 0{,}8 \cdot 0{,}375 \\ 0{,}2 \cdot 0{,}85 \cdot 0{,}75 + 0{,}85 \cdot 0{,}8 \cdot 0{,}375 \end{pmatrix} = \begin{pmatrix} \ldots \\ 0{,}55 \\ 0{,}06 \\ 0{,}38 \end{pmatrix} \begin{array}{c} j \\ 8 \\ 9 \\ 10 \end{array} \end{array}$$

ce qui est en bon accord avec les valeurs expérimentales de $\widetilde{G}'_4$. Dans le cas des réactions à $n = 5$, le rendement radiolytique des produits en C_5 est trop faible pour pouvoir faire l'objet d'un calcul précis. De toute manière, le seul pic intense du spectre de masse est $C_5H_9^+$, et il lui correspond en radiolyse un seul produit en C_5, le n-pentane qui peut se former selon (2) à partir de ce dernier ion.

Il semble en résumé que, malgré son caractère peu élaboré, le modèle semi-empirique présenté ci-dessus permette de relier, au moins en première approximation, les résultats de radiolyse de ceux de spectrométrie de masse.

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*Absolute Konfiguration von Xanthophyll (Lutein)**

Summary

The chirality of xanthophyll (lutein) has been shown by chemical degradation and application of chiroptical methods to be 3R, 3'R, 6'R.

Alle α-Carotinderivate, deren absolute Konfiguration bis heute bestimmt werden konnte, nämlich δ-Carotin[1], α-Carotin[2], Semi-α-carotinon[3], ε-Carotin[1,2], Zeinoxanthin (= α-Cryptoxanthin)[4] und Crocoxanthin[4] weisen im α-Ring (C-6 bzw. C-6') identische Konfiguration (R) auf.

Das wichtigste hydroxylierte α-Carotin in höheren Pflanzen ist Xanthophyll (= Lutein) (I). Wir haben die Konfiguration seiner drei chiralen Zentren wie folgt festlegen können:

a) *Zentrum C-6'*[5]: Oxydation von Xanthophyll mit NiO_2 und nachfolgende Destillation und Chromatogra-

* Eingegangen am 13. Mai 1971.

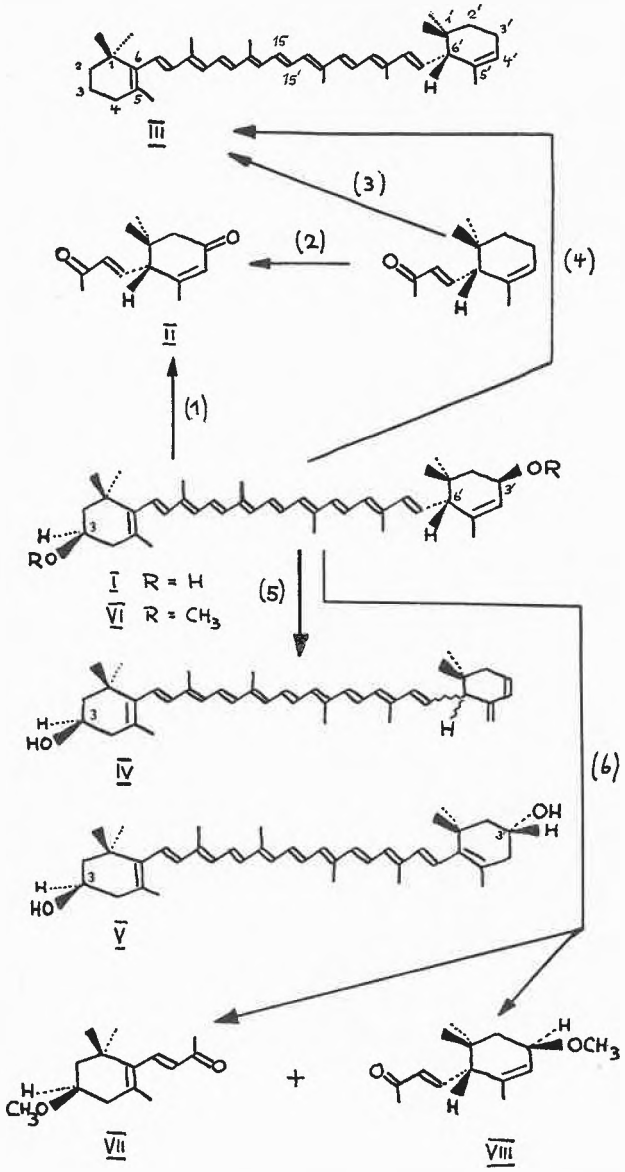
[1] R. BUCHECKER und C. H. EUGSTER, *Helv. Chim. Acta 54* (1971) 327.

[2] C. H. EUGSTER, R. BUCHECKER, CH. TSCHARNER, G. UHDE und G. OHLOFF, *Helv. Chim. Acta 52* (1969) 1729.

[3] R. BUCHECKER, H. YOKOYAMA und C. H. EUGSTER, *Helv. Chim. Acta 53* (1970) 1210.

[4] L. BARTLETT, W. KLYNE, W. P. MOSE, P. M. SCOPES, G. GALASKO, A. K. MALLAMS, B. C. L. WEEDON, J. SZABOLCS und G. TÓTH, *J. Chem. Soc. (C) 1969*, 2527.

[5] C. H. EUGSTER, *Angew. Chem. 1970*, 259.

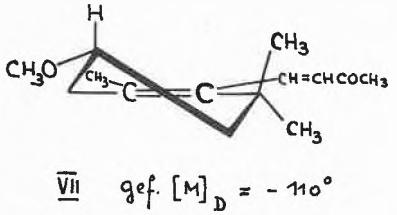


Reaktionsschritte: (1) NiO_2, Benzol/Äther (1:1), 2 bis 3 Tage schütteln bis zur Entfärbung; (2) *t*-Butylchromat; (3) siehe [6]; (4) Ditosylat, $LiAlH_4$ in Äther/Benzol; (5) $LiAlH_4 + AlCl_3$ in Benzol/Äther (1:1); (6) O_2, $h\nu$, Rose Bengale oder NiO_2, Benzol/Äther (1:1), bis zur Entfärbung

phie der ketonischen Produkte gab kristallines (+)-3-Oxo-α-jonon (II), ($[\alpha]_D^{24} = +235°$ in Alkohol; λ_{max} 237,5 nm, $\varepsilon = 19300$, in Alkohol; CD in Alkohol: 322 nm ($\Delta\varepsilon = -1{,}88$), 240 nm ($\Delta\varepsilon = +35{,}9$); IR ($CCl_4$): 1702 s, 1676 s, 1622 s cm^{-1}). Dessen Chiralität ergab sich aus der Tatsache, daß (−)-α-Jonon, das seinerseits über eindeutige Schritte mit (−)-α-Carotin (ent-III) verknüpft ist[6], bei der Oxydation mit *t*-Butylchromat[7] kristallines, partiell racemisiertes (−)-3-Oxo-α-jonon (ent-II) liefert ($[\alpha]_D^{24} = -5{,}2°$ in Alkohol; λ_{max} 237,5 nm ($\varepsilon = 21200$), in Alkohol; CD in Alkohol ($\Delta\varepsilon$): 322

[6] Ch. Tscharner, C. H. Eugster und P. Karrer, *Helv. Chim. Acta 40* (1957) 1676.

[7] V. Prelog und M. Osgan, *Helv. Chim. Acta 35* (1952) 986.



VII gef. $[M]_D = -110°$

Abb. 1

nm (+0,056), 239 nm (−1,53 ± 0,5); IR (CCl_4) identisches Spektrum).

Ferner ließ sich (+)-Xanthophyll durch Reduktion seines Ditosylates in allerdings sehr geringer Ausbeute in (+)-α-Carotin (III) überführen.

Damit ergibt sich die Chiralität von (+)-Xanthophyll an C-6′ zu R.

b) *Zentrum C-3:* Wenn man (+)-Xanthophyll mit $LiAlH_4/AlCl_3$ behandelt, so tritt Elimination der allylischen C-3′-Hydroxylgruppe und Racemisierung an C-6′ ein. Das neue Carotinoid hat Struktur IV (Smp. 160°, λ_{max} in Dioxan (ε): 481 (127000), 453 (142000), 428 (94500), 331 (11600), 267 (34800) nm; sein CD stimmt mit 335 ($\Delta\varepsilon + 2{,}3$), 279 (−14,2), 244 (+10,7), 227 (−8,0), 213 (+8,15) nm gut mit den Werten von Zeaxanthin (V), jedoch mit verminderten $\Delta\varepsilon$-Werten, überein. Seine Chiralität konnte durch oxydativen Abbau am (+)-Xanthophylldimethyläther (VI) zu (−)-3-Methoxy-β-jonon (VII) und (+)-3-Methoxy-α-jonon (VIII) bestimmt werden. Das durch präparative Gaschromatographie abgetrennte VII ($[\alpha]_D^{24} = -33° \pm 4°$ in Alkohol; λ_{max} 289 ($\varepsilon = 8070$), 219 (8020), 209 (7470) nm, in Alkohol; CD in Alkohol: 347 ($\Delta\varepsilon + 0{,}01$), 320 (−0,01), 282 (+0,05) nm; IR (CCl_4): 1694 s, 1675 s, 1608 s cm^{-1}) kann durch Anwendung der Millsschen Regel[8], siehe Abb. 1, konfigurativ festgelegt werden. Demnach hat das Zentrum an C-3 R-Chiralität.

c) *Zentrum C-3′:* Das in b) erwähnte (+)-3-Methoxy-α-jonon (VIII) wurde ebenfalls durch Gaschromatographie rein erhalten ($[\alpha]_D^{24} = +288° \pm 4°$ in Alkohol; λ_{max} 225 nm ($\varepsilon = 13000$), in Alkohol; CD ($\Delta\varepsilon$) 368 (−0,12), 352 (−0,37), 338 (−0,58), 325,5 (−0,6), 315 (−0,50), 233 (+20,2) nm, in Methylcyclohexan-iso-Pentan (3:1). Es enthält zwei Chiralitätszentren, nämlich das ursprüngliche C-6′ und C-3′ des Xanthophylls. Durch NMR-Analyse[9] ließ sich zeigen, daß Butenonseitenkette und Methoxyl am Cyclohexenring *trans* stehen. Zentrum C-3′ des Xanthophylls hat demnach R-Konfiguration, und Xanthophyll ist 3R, 3′R, 6′R.

Weedon *et al.*[10] haben kürzlich die absolute Konfiguration des (+)-Xanthophylls zu 3R, 3′S, 6′R angegeben. 3R folgte aus der Verknüpfung mit Zeinoxanthin und Zeaxanthin, 6′R aus der chiroptischen Korrelation mit

[8] E. L. Eliel, *Stereochemistry of Carbon Compounds*, McGraw-Hill, London 1962, S. 410.

[9] Die vollständige NMR-Analyse wird in der ausführlichen Arbeit wiedergegeben werden.

[10] D. Goodfellow, G. P. Moss und B. C. L. Weedon, *Chem. Comm. 1970*, 1578.

α-Carotin[2], und 3′S wurde ohne chemischen Beweis aufgrund biogenetischer Überlegungen angenommen[11].

Unsere auf unabhängigem Wege gewonnen Ergebnisse stimmen mit denen von WEEDON *et al.*[10] bezüglich der Zentren C-3 und C-6′ überein. Sie bestätigen die Brauchbarkeit des ORD-Additionsverfahrens bei Carotinoiden[4]. Der Widerspruch bezüglich des Chiralitätszentrums C-3′ könnte darauf zurückzuführen sein, daß in unserem Methylierungsschritt (K-*t*-Butylat, *t*-Butanol/Benzol, CH_3I) eine Konfigurationsumkehr eingetreten ist. Wir glauben, dies ausschließen zu können, da einerseits bei Verwendung von deuteriertem *t*-Butanol kein D in Xanthophylldimethyläther eingebaut wird und anderseits Methylierung mit Ag_2O, Chloroform, CH_3I oder BaO, DMF/DMSO, CH_3I[12] (unter Sauerstoffausschluß) stets zum selben Methoxy-α-jonon (VIII) geführt hat. Diese Ergebnisse machen eine Umlagerung am C-3′ während der Methylierung wenig wahrscheinlich, schließen sie jedoch noch nicht streng aus.

Wir danken dem Schweizerischen Nationalfonds (Projekte Nr. 2.113.69 und 5011.2) für finanzielle Unterstützung, der Firma Firmenich S.A., Genève, für Hilfe bei der gaschromatographischen Trennung, Herrn Professor W. v. PHILIPSBORN für seine Ratschläge bei der NMR-Analyse.

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[11] T. J. WALTON, G. BRITTON und T. W. GOODWIN, *Biochem. J. 112* (1969) 383.

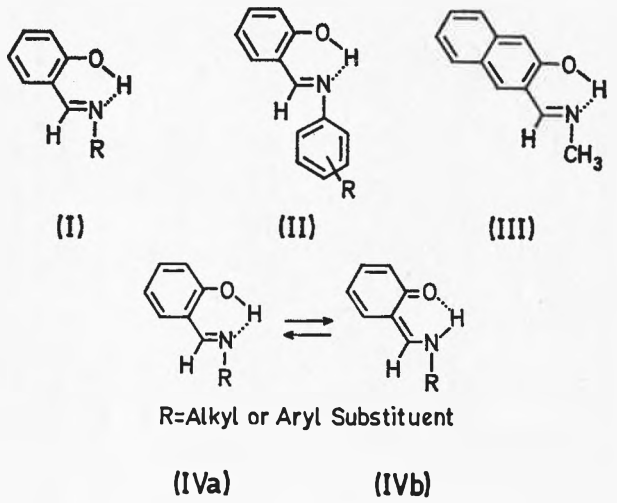
[12] H. MÜLLER und P. KARRER, *Helv. Chim. Acta 48* (1965) 291.

Infrared and Proton Magnetic Resonance Spectra of N-Alkyl and N-Aryl Salicylaldimines *

Summary

The infrared spectra of nine N-alkyl and twentyfour N-aryl salicylaldimines have been determined. ^{15}N-Labelling of N-*p*-tolylsalicylaldimine yields assignments of the more significant vibrational bands and enables an assessment of the extent of vibrational coupling of $\nu C{=}N$. The proton magnetic resonance spectra of the salicylaldimines show that they exist in the phenolimine form at room temperature in deuterochloroform. Electron withdrawing substituents reduce the magnetic shielding of the hydroxyl proton by modifying the capacity of the nitrogen atom for entering into hydrogen bonding.

In view of extensive vibrational coupling, there is considerable difficulty in making reliable empirical assignments in the infrared spectra of SCHIFF bases (I, II) derived from the condensation of salicylaldehyde with aliphatic and aromatic amines. ^{15}N-Labelling provides a suitable approach[1] to the assignment problem and enables the relative purity of the vibrational bands to be estimated. Here we report the assignments of the more significant vibrations in the i.r. spectra which result from ^{15}N-labelling of the compound (II; R = 4-CH_3) and the influence of varying the substituents R on the



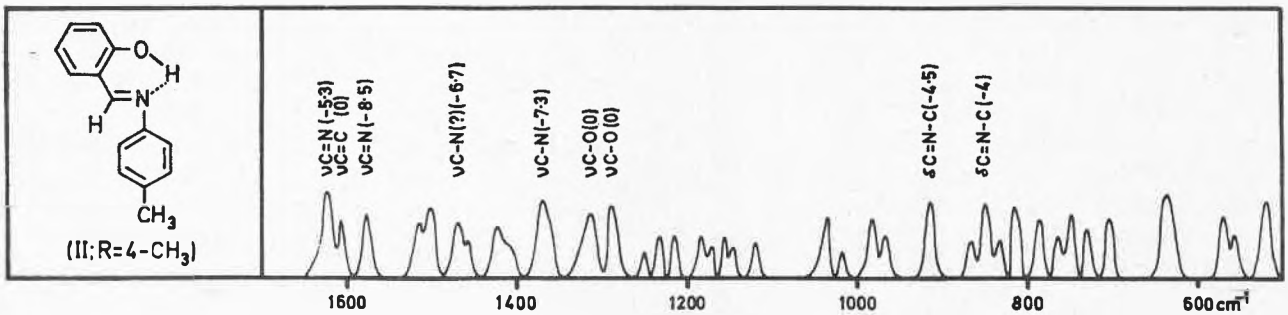
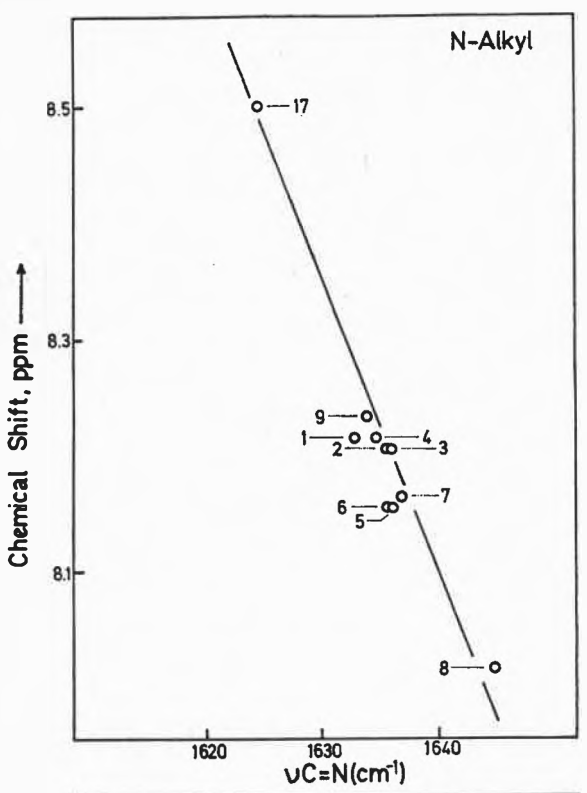


Figure 1. Infrared spectrum of N-*p*-tolylsalicylaldimine with assignments. Figures in parenthesis are ^{15}N-induced shifts (cm^{-1}). All other bands are shifted < 1.5 cm^{-1}. Sampling as nujol mulls except for 1340 to 1480 cm^{-1} region (as hexachlorobutadiene mulls)

* Received Mai 14, 1971.

[1] G. O. DUDEK, *J. Org. Chem. 32* (1967) 822.



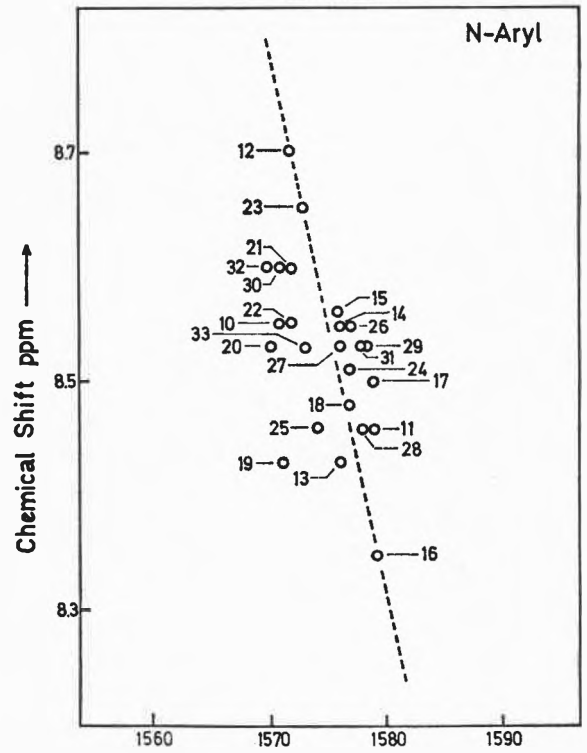
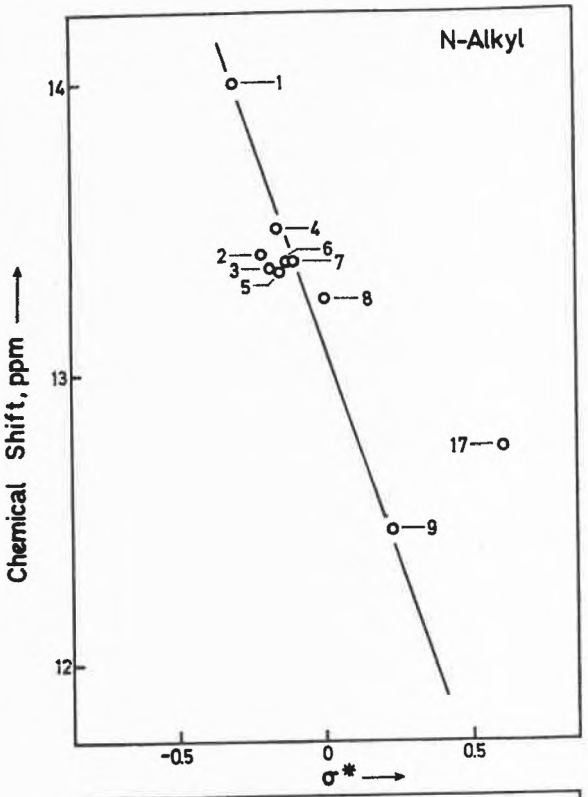


Figure 2. Relationship between downfield chemical shifts of methine proton (in $CDCl_3$, relative to TMS) and the lesss coupled $\nu C{=}N$ for N-alkyl and N-aryl salicylaldimines



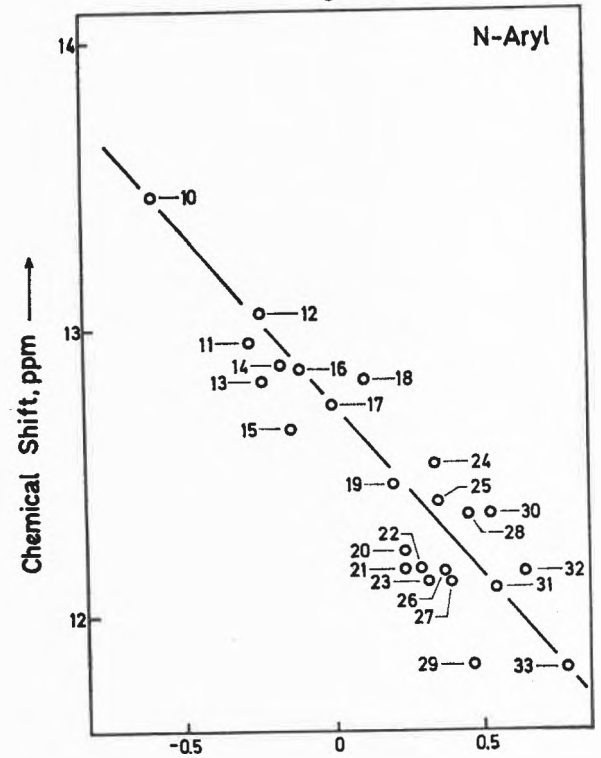


Figure 3. Relationship between downfield chemical shift of hydroxyl proton (in $CDCl_3$, relative to TMS) and TAFT (σ^*) or HAMMETT (σ) substituent parameters of substituted N-alkyl and N-aryl salicylaldimines

i.r. and p.m.r. spectra of nine N-alkyl and twenty-four N-aryl salicylaldimines**.

** Key to substituents (Figs. 2 and 3): N-Alkyl compounds (I): *1*, *t*-C_4H_9; *2*, *s*-C_4H_9; *3*, *i*-C_3H_7; *4*, *cyclo*-C_6H_{11}; *5*, *n*-C_4H_9; ***6***, *i*-C_4H_9; *7*, *n*-C_3H_7; *8*, CH_3; *9*, $C_6H_5CH_2$. – N-Aryl compounds (II): *10*, 4-$N(CH_3)_2$; *11*, 4-OCH_3; *12*, 4-OC_2H_5; *13*, 3,4-di-CH_3; *14*, 4-CH_3; *15*, 4-C_2H_5; *16*, 3-CH_3; *17*, H; *18*, 3-OCH_3; *19*, 3-Cl, 4-CH_3; *20*, 4-Cl; *21*, 4-Br; *22*, 4-I; *23*, 3-$COCH_3$; *24*, 3-F; *25*, 3-I; *26*, 3-Cl; *27*, 3-Br; *28*, 4-$Co_2C_2H_5$; *29*, 4-COC_6H_5; *30*, 4-$COCH_3$; *31*, 3-NO_2, 4-CH_3; *32*, 4-CN; *33*, 3,5-di-Br.

Figure 1 shows that three bands are observed in the νC=N region (1570 to 1630 cm^{-1}) of N-*p*-tolylsalicylaldimine (II; R = 4-CH_3) but the central band at 1605 cm^{-1} is insensitive to both ^{15}N-substitution and to substitution in the N-aryl ring. These observations and its position suggest its assignment to the aromatic ring stretching frequency normally fount near 1600 cm^{-1}. The two outer bands are sensitive to both ^{15}N and N-aryl substitution and are accordingly assigned to coupled νC=N. Only the higher frequency band has been[2] empirically assigned to this vibration but the present work establishes, firstly, that both bands are extensively coupled (the observed ^{15}N-induced shifts are −5 and −8 cm^{-1} compared with −40 cm^{-1} expected[3] for uncoupled νC=N) and, secondly, that the lower frequency band probably has the greater vibrational purity.

Conjugation of the N-phenyl group with the heterocyclic ring in the N-aryl bases (II) is expected to lead to more extensive vibrational coupling than exists in the N-alkyl compounds (I). Accordingly, ^{15}N-labelling of the N-methyl compound (III) has been found[1] to induce a shift of −18 cm^{-1} in the higher frequency band and negligible shift in the lower frequency band. Furthermore, since proton chemical shifts are generally proportional to the *p*-electron densities on the atoms to which they are bound, we would expect increased shielding of the methine proton to accompany any substituent-induced increase in νC=N. The extent to which the expected correlation is observed will clearly depend on the vibrational purity of νC=N. Thus, if the less-coupled νC=N band from the spectra of the N-alkyl and N-aryl compounds is compared with the methine proton shielding, a better correlation is expected for the N-alkyl than the N-aryl compounds. This is observed (Fig. 2).

No assignments have previously been proposed for νC–N in compounds of the type discussed here (we distinguish the C=N of the heterocyclic ring from the exocyclic C=N bond by using double and single bonds, respectively). A band at 1370 cm^{-1} in the hexachlorobutadiene mull spectrum of (II; R = 4-CH_3) is shifted −7 cm^{-1} by ^{15}N-substitution and is reasonably assigned to νC–N. An additional band at 1464 cm^{-1}, similarly shifted, may have similar origin but the latter frequency seems rather high for νC–N. The band at 1286 cm^{-1} in (II; R = 4-CH_3) has been empirically assigned[2] to the phenolic νC–O. As its frequency is unchanged by ^{15}N-labelling but increased by metal ion complexation[2] this assignment is probably correct. νO–H, near 2700 cm^{-1}, is broadened by hydrogen bonding to an extent which precludes precise evaluation of its frequency, nor is its definition significantly improved in dilute solution spectra.

The p.m.r. spectra of salicylaldimines and related compounds have previously been chiefly employed to examine tautomeric equilibria. While aliphatic β-keto-amines exist predominantly in the keto-amine form[4], it has been established[5] that the compound (II; R = H) exists solely in the phenol-imine form (IVa) with no p.m.r. evidence for the keto-amine form (IVb) at room temperature in chloroform. A similar conclusion is reached for all the compounds studied here. Except for very small splitting of the methine and methyl proton signals ($J \sim 1$ cps) in the spectrum of (I; R = CH_3), none of the signals arising from the protons on the carbon atoms adjacent to the heterocyclic nitrogen exhibits any doublet character.

The electronic effects of the substituents R may be transmitted to affect the intramolecular hydrogen bonding either *via* the conjugation of the heterocyclic ring or, more directly, by modifying the capacity of the nitrogen atom for entering into hydrogen bonding. Since some bond fixation is implied by predominance of the tautomer (IVa), the latter route would appear more likely. The fact that neither the methine proton shielding nor νC=N exhibits any regular correlation with the HAMMETT (σ) or TAFT (σ^*) substituent parameters while the hydroxyl proton shielding does exhibit reasonable correlations with σ and σ^* (Fig. 3) is in support of the more direct route. Electron withdrawing substituents will decrease the electron density on the nitrogen atom. The consequent reduction in the N–H bonding will increase the O–H bond strength and hence increase the shielding of the hydroxyl proton. This is observed. Certainly, the substituent effect is more consistent with (IVa) than (IVb) as the predominant species. If the tautomeric equilibrium favoured (IVb), electron releasing substituents would increase the shielding of the proton now primarily resident on the nitrogen atom. This does not occur.

Experimental

Infrared spectra were determined on a Beckman IR-12 spectrophotometer on nujol mulls (N-aryl salicylaldimines and N-benzyl salicylaldimine) or liquid films (N-alkyl salicylaldimines) between caesium bromide plates. The region masked by nujol absorption was determined on hexachlorobutadiene mulls. The p.m.r. spectra were determined on a Varian A-60 spectrometer in deuterochloroform at 17 to 23 °C (internal standard: tetramethylsilane). Purity of all compounds was established by microanalysis.

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