Formation of Charge-Transfer Complexes of Penicillins with Iodine

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Benzylpenicillin, benzathine benzylpenicillin, procaine benzylpenicillin, anhydrous ampicillin and sodium ampicillin form charge-transfer complexes with iodine in 1,2-dichloroethane, which were examined by UV, FIR, ^{1}H-NMR, ^{13}C-NMR, and ^{15}N-NMR spectroscopy.

Bildung von Charge-Transfer Komplexen von Penicillinen mit Iod

Benzylpenicillin, Benzylpenicillin-Benzathin, Benzylpenicillin-Procain, wasserfreies Ampicillin und Ampicillin-Natrium bilden nach UV-, FIR-, ^{1}H-NMR-, ^{13}C-NMR- und ^{15}N-NMR-Untersuchungen mit Iod in 1,2-Dichlorethan Charge-Transfer Komplexe.

In 1981, *Belal* et al.[1] described the determination of benzylpenicillin (**1**) through formation of charge-transfer complexes (CTC) with chloranil. CTC formation of penicillines with iodine has not been reported.

The violet colour of iodine in 1,2-dichloroethane solution changes immediately to lemon yellow upon addition of penicillins[2]. Two new bands at 293 and 363 nm appear in the UV range[3]. The molar absorptivities and association constants for the benzylpenicillin (**1**), benzathine benzylpenicillin (**2**), procain benzylpenicillin (**3**), anhydrous ampicillin (**4**) and sodium ampicillin (**5**) iodine interaction products were evaluated using the *Benesi-Hildebrand* equation (Tab. 1)[2], whereby the concentration of the donor is higher than that of the acceptor[4,5]. The high values of the association constants (Tab. 1) are common to complexes between σ-acceptors, such as iodine and n-donors. Application of the *Job's* molar variation method[6] results in an equimolar ratio, for **2** and **3** and a ratio of 2:1 (donor:I_2) for **1**, **4**, and **5**[2].

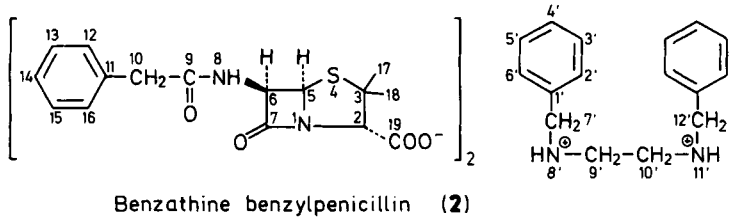
Penicillin degradation products such as benzylpenicilloic acid, 6-aminopenicillanic acid, penicillamine and benzylpenicillenic acid were also found to interact with iodine under the same reaction conditions.

It was not possible to isolate complexes of **1** to **5** with iodine by the usual methods. Therefore, the preparations formed by mixing the two components using a mortar and pistle according to lit.[7], **[1a-5a]**, were used for the FIR, ^{1}H-NMR, ^{13}C-NMR and ^{15}N-NMR spectroscopic analysis.

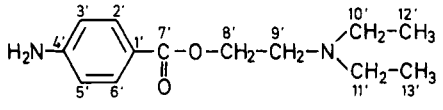
In the far infrared spectrum, the preparation of **1** with iodine **[1a]** shows an absorption at 138 cm^{-1} which could be assigned to the charge transfer complex[8]. This band is not found in the spectra of either **1** or iodine.

Tab. 1: Molar Absorptivities and Association Constants of Charge Transfer Complexes of Penicillines with Iodine in 1,2-Dichloroethane[3]

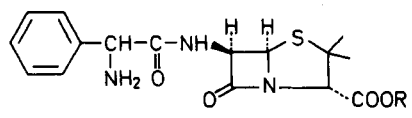
Donor	Donor-Iodine Complexes	
	Absorptivity $\cdot 10^5$ (293 nm)	Association constant $\cdot 10^3$ [1 mole^{-1}]
1	2.08	1.6
2	1.17	1.35
3	20 (363 nm)	6.55
4	65.4	2.76
5	2.15	1.69



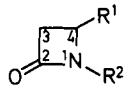
Benzathine benzylpenicillin (**2**)



Procaine



4: R=H, Anhydrous ampicilline
5: R=Na, Sodium ampicillin



6: R^1, R^2 = H, Azetidin-2-one
7: R^1, R^2 = C_6H_5, Diphenyl-azetidin-2-one

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Tab. 2: ^{1}H-NMR Spectra (d_6-DMSO) of Benzylpenicillin (**1**), Benzathine benzylpenicillin (**2**), Procaine benzylpenicillin (**3**), anhydrous Ampicillin (**4**), Sodium Ampicillin (**5**) and their Iodine Preparations **1a** - **5a**

	Chemical Shifts (δ-ppm)									
Proton	**1**	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5**	**5a**
Me-17	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.5	1.4
Me-18	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.6	1.6
10-H	3.6	3.6	3.6	3.6	3.6	3.6	4.0	4.1	4.5	4.8
2-H	4.0	4.2	4.1	4.2	4.1	4.2	4.8	5.0	4.0	4.1
5,6-H	5.4	5.5	5.4	5.5	5.4	5.5	5.4	5.5	5.4	5.5
Ar-H	7.3	7.3	7.5	7.5	7.5	7.3	7.4	7.4	7.4	7.3
8-H	8.8	8.8	9.1	9.5	8.8	8.8	9.0	9.1	8.7	9.0
10-NH_2							6.3	6.7	3.3	4.2
9',10'-H			3.0	3.3						
7',12'-H			3.9	4.2						
8',1'-H			5.3	6.2						
2'H to 6'H			7.4	7.5						
Me-12',13'					1.1	1.2				
10',11'-H					2.8	3.1				
9'-H					3.1	3.3				
8'-H					4.3	4.4				
4'-NH_2					5.7	5.7				
2',6'-H					6.6	6.6				
3',5'-H					7.8	7.8				

Tab. 3: ^{13}C-NMR spectra (d_6-DMSO) of the Iodine Preparations of Penicillins **1a** - **5a** and their Differences ($\Delta\delta$) from that of the Non-complexed Derivatives **1-5** (+: downfield, -: upfield shift).

C-	Chemical Shifts (δ-ppm)									
Atom	**1a**	$\Delta\delta$	**2a**	$\Delta\delta$	**3a**	$\Delta\delta$	**4a**	$\Delta\delta$	**5a**	$\Delta\delta$
19	173.6	-0.5	173.7	+0.3	173.6	+0.3	172.6	-0.3	173.6	-0.1
9	170.6	-0.5	170.5	0	170.5	+0.1	167.8	-2.2	172.6	-0.7
7	169.1	-1.6	169.1	-1.2	169.4	-0.4	169.2	-0.5	170.3	-0.1
11	135.7	-0.4	135.9	0	135.9	0	129.9	-7.2	141.0	-1.0
12;16	129.1	-0.2	129.9	-0.7	129.1	0	128.7	+0.4	128.5	+0.4
13;15	128.2	0	129.2	0	128.9	0	127.7	+0.3	127.7	+0.6
14	126.5	0	126.5	0	128.6	0	127.7	+0.3	127.4	+0.5
2	70.4	-3.6	70.4	-1.9	71.0	+0.2	70.7	-1.6	73.5	-0.4
5	67.4	+0.4	67.3	+0.1	67.3	+0.1	66.7	-0.1	66.8	0
3	63.9	-0.5	63.8	-0.3	63.9	0	63.7	-0.3	64.3	-0.1
6	58.6	+0.8	58.5	+0.3	58.4	+0.2	58.0	+0.3	57.8	+0.6
10	41.5	-0.1	41.4	0	41.4	0	55.1	+1.0	57.5	+0.4
17	30.6	-0.4	30.3	-0.7	30.5	-0.3	26.7	-0.5	31.4	-0.2
18	26.8	-0.8	26.7	-0.4	26.8	-0.1	30.3	+3.3	27.3	-0.1
1'			131.9	-3.6						
2',6'			128.9	+0.3						
3',5'			128.5	+0.3						
4'			128.5	+0.5						
7',12'			50.4	-0.5						
9',10'			42.6	-1.8						
7'					165.5	0				
4'					153.9	+0.2				
2',6'					131.4	+0.3				
1'					115.1	-0.3				
3',5'					112.7	0				
9'					58.7	+0.6				
8'					49.8	0				
10',11'					47.1	+0.5				
12',13'					8.9	-1.1				

In the ^{1}H-NMR spectra (d_6-DMSO) (Tab. 2) the 2-H, 5-H and 6-H signals are shifted to lower field ($\Delta\delta$ = 0.1-0.2 ppm). In **2a**, shifts were also observed in the methylene and the NH protons of the benzathine part ($\Delta\delta$ = 0.3 and 0.9 ppm). In **3a**, the aliphatic protons of the methyl and methylene groups in the procaine part are also downfield shifted ($\Delta\delta$ = 0.1 and 0.3 ppm). In **4a** and **5a**, 2-H and 10-H as well as 8-H and 10-NH_2 are shifted to

lower field ($\Delta\delta$ = 0.1-0.9 ppm). The chemical shift difference of 10-NH_2 of **5a** ($\Delta\delta$ = 0.9 ppm) is greater than that obtained in the case of **4a** ($\Delta\delta$ = 0.4 ppm). This might be attributed to the zwitter ion formation in the case of **4**.

The ^{13}C-NMR spectrum of **1a** (Tab. 3) shows a downfield shift of C-5 and C-6 ($\Delta\delta$ = 0.4 and 0.8 ppm) against an upfield shift of $\Delta\delta$ = -3.6, -1.6, -0.4, -0.8, -0.5, -0.5, -0.4, and -0.5 ppm for C-2, C-7, C-17, C-18, C-19, C-3, C-11 and C-9 in comparison with **1**. Compared with **2** and **3**, the benzylpenicillin part of **2a** and **3a** reveals both down and upfield shifts which are similar to that of benzylpenicillin sodium salt[3]. In the benzathine moity, downfield shifts of C-2', C-6', C-3', C-5' and C-4' occur and upfield shifts of C-1', C-7', C-12', C-9' and C-10'. The procaine part reveals upfield shifts in C-1', C-12' and C-13' in addition to downfield shifts in C-2', C-6', C-4', C-9', C-10' and C-11'. Salt formation and interaction with iodine seem to change the polarization especially of C-1'. Accordingly, this atom in the free benzathine base (δ = 141.2 ppm) is shifted to δ = 135.5 ppm on formation of benzathine penicillin salt, whereas it is shifted to δ = 131.9 ppm on interaction with iodine.

In the ^{15}N-NMR spectrum (d_6-DMSO) of **1a**, N-1 is shifted upfield to δ = 160.1 ppm by 7 ppm from that of free **1** (δ = 167.2 ppm) while N-8 is scarcely affected (δ = 112.4 ppm to 111.9 ppm)[2]. As reported[9,10], anomalous upfield shifts in ^{15}N-NMR (brought about during interaction of the N-atom with electron acceptors) can be explained on the basis of electronic excitations involving the antibonding molecular orbitals with a subsequent shielding effect on the two atoms involved in the bond.

The UV maxima at 293 and 363 nm as well as the FIR absorption at 138 cm^{-1}, give evidence for interaction of iodine as σ-acceptor with penicillins as n-donors[3,11]. Further information is given by the NMR spectra although they were registered in d_6-DMSO and not in 1,2-dichloroethane due to the low solubility of the preparations **1a-5a** in this solvent. In the ^{1}H-NMR spectrum of **1a**, 2-H is more downfield affected than 5-H and 6-H. It can, therefore, be suggested that the lactam nitrogen N-1 or the oxygen of the lactam carbonyl is the donor center. From the ^{13}C- and ^{15}N-NMR spectra, N-1 is in favour. Thus, in the ^{13}C-NMR spectrum, C-2 is most affected[12]. The upfield shift of the ^{15}N-NMR signal of N-1 also demonstrates participation of the lactam -N in a charge-transfer process[13].

Further two β-lactam derivatives, azetidin-2-one (**6**) and 1,4-diphenylazetidin-2-one (**7**) were reacted with iodine under the same reaction conditions. **6** was found to demonstrate the 293 and 363 nm bands, while no such bands are observed for **7**. Apparently, a critical electron density is required for the nitrogen atom in the β-lactam structure.

Experimental Part

UV/VIS: Spectrophotometer 550 S (Perkin Elmer). - FIR: Spectrometer FS, 114 C (Bruker). - ^{1}H-, ^{13}C- and ^{15}N-NMR: Spectrometer EM-360 A (Varian), WH-90 (Bruker Physics) and XL-300 (Varian). - The FIR spectra were measured as polyethylene tablets.

Stoichiometric Relationship[14]

Equimolar solutions of I_2 in 1,2-dichloroethane and the diverse penicillin preparations in 1,2-dichloroethane (dissolved in 0.5% of DMSO) were prepared: 1.0 x 10^{-4} M for **2**, **4**, and **5**, 0.7 x 10^{-4} M for **1** and 0.5 x 10^{-4} M for **3**. A series of 10 ml quantities of mixtures containing a total of 10 ml of the solutions in different complementary proportions (from 0:10 to 10:0 inclusive) were made up in 10 ml volumetric flasks, which were allowed to stand at 25 ± 1°C for the specified time. Absorbances were measured at λ = 363 nm for **3**-iodine and at λ = 293 nm for the other compounds.

Association Constants and Molar Absorptivities[5]

Pencillin solutions were prepared in 1,2-dichloroethane (after dissolving in 1.0 % DMSO) as follows: 0.4, 0.8, 1.2, 1.6, and 2.0 x 10^{-4} M solutions and 2.5 x 10^{-5} M I_2-solution. The penicillin solution and the I_2-solution were placed in a water-bath at 25 ± 1°C for 30 min. 5.0 ml of the I_2-solution were quickly mixed with the same volume of each penicillin solution. The absorbance was determined immediately at λ = 363 nm for **3** and at λ = 293 nm for the other compounds.

Penicillin-Iodine Samples for Spectroscopic Investigations

According to the obtained molar ratio, accurately weighed quantities of penicillin and I_2 were mixed using mortar and pistle according to lit.[7].

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References

1 S. Belal, M.A. El-Sayed, M.E. Abdel-Hamid, and H. Abdine, J. Pharm. Sci. *70*, 127 (1981).
2 G.A. Saleh, Ph. D. Thesis, Assiut University, Egypt.
3 W. Knoll, Elektronen-Donor-Aceptor-Komplexe aliphatischer Amine mit Iod: Struktur und Verwendung zur Detektion in der HPLC, Ph. D. Thesis, Tübingen, W.-Germany, 1986.
4 R. Foster, Organic Charge Transfer Complexes, Academic Press, London and New York 1969.
5 H.A. Benesi and J.H. Hildebrand, J. Am. Chem. Soc. *71*, 2703 (1949).
6 H.H. Jaffé and M. Orchin, Theory and Applications of Ultraviolet Spectroscopy, p.581, John Wiley & Sons, Inc., New York and London 1962.
7 M. Slifkin, Charge Transfer Complexes, p. 47, Academic Press, London and New York 1971.
8 A. Finch, P.N. Gate, F.N. Diekson and F.F. Bently, Chemical Application of Far Infrared Spectroscopy, Academic Press, London and New York 1970.
9 N. Juranic and R. Lichter, J. Am. Chem. Soc. *105*, 406 (1983).
10 D. Cornwell, J. Chem. Phys. *44*, 874 (1966).
11 A.M. Taha and C.S. Gomaa, J. Pharm. Sci. *65*, 986 (1976).
12 K.A. Kovar and M. Abdel-Hamid, Topics in Pharmaceutical Sciences; D.D. Breimer and P. Speiser (ed.), p. 15, Elsevier Sciences Publishers, Amsterdam 1983.
13 R.N. Someswara, D.M. Mariya, R.G. Babu, and Z. Dieter, Spectrosc. Lett. *18*, 827 (1985).
14 J. Rose, Advanced Physico-chemical Experiments, Pittmann Verlag, London 1964.

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