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THE CONVERSION OF BETANIDIN AND BETANIN TO NEOBETANIDIN DERIVATIVES[1]

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Abstract—Details are given for the conversion of betanin, the pigment of the red beet, and its aglucone, betanidin, to a number of derivatives of neobetanidin trimethyl ester. The conversion occurred, presumably by air-oxidation, when the basic conjugated system was deprotonated under the influence of diazomethane, pyridine and acetic anhydride, amines or sodium acetate.

The neobetanidin-esters turned out to be 14,15-dehydrobetanidin derivatives and were the key for the structure elucidation of the betacyanins and betaxanthins. Their constitutions could be derived essentially by comparison of the NMR spectrum of the 5,6-di-O-acetyl derivative with those of two degradation products from betanidin, namely N-acetyl-5,6-diacetoxy-2,3-dihydroindole-2-carboxylic-acid methyl ester (now called triacetylcyclodopa methyl ester) and 4-methyl-pyridine-2,6-dicarboxylic-acid methyl ester.

A separate proof for the vinylene group connecting the two heterocycles was available through its reduction in a Pd catalyzed reaction at the expense of a dehydrogenation of the dihydroindole system. The same type of disproportionation reaction could also be applied to N-styrylindolin, which was converted to N-(β-phenylethyl) indole.

The neobetanidin structures contain a "1,7-diazaheptamethin" system. This new expression, which is explained briefly, allows a rationalization of the absorption spectra (color), the halochromism (about 100 nm), the pK_a-values (1·6 to 2·4) and certain NMR signals of the neobetanidin derivatives.

INTRODUCTION

THE red-violet water-soluble pigments characteristic for ten plant families[3] of the order of Centrospermae are called betacyanins.[4] A prominent member of this group is betanin, found abundantly in the red beet,[5] in many cacti[6,7] and in the pokeberry.[8] The hydrolysis of all betacyanins studied so far leads to two isomeric aglycones, betanidin and isobetanidin.[6,9] The established[1,10] structure of betanidin is represented

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[1] A preliminary account of a part of this work was previously presented: T. J. Mabry, H. Wyler, G. Sassu, M. Mercier, I. Parikh and A. S. Dreiding, *Helv. Chem. Acta* **45**, 640 (1962).

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[2b] In part from the dissertation of I. Parikh, University of Zurich (1966). Monsanto predoctoral fellow 1962/63.

[3] For a recent review of the phylogenetic significance of the betacyanin-betaxanthin pigments with respect to the Centrospermae families, see T. J. Mabry, *The Betacyanins and Betaxanthins* Chap. in *Comparative Phytochemistry* (Edited by T. Swain) Academic Press, London (1966).

[4] A. S. Dreiding, *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Edited by W. D. Ollis) p. 194. Pergamon Press, London (1961).

[5] H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **40**, 191 (1957).

[6] H. Wyler and A. S. Dreiding, *Experientia* **17**, 23 (1961).

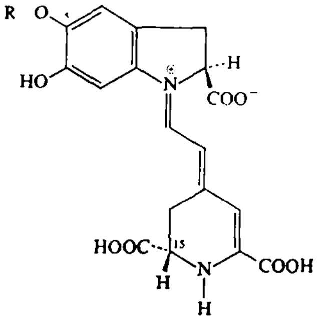
[7] M. Piatelli and L. Minale, *Phytochemistry* **3**, 307 (1964).

[8] H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **44**, 249 (1961).

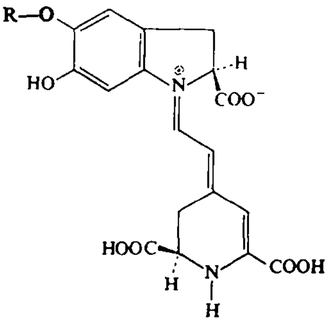
[9] M. Piatelli and L. Minale, *Phytochemistry* **3**, 547 (1964).

[10] H. Wyler, T. J. Mabry and A. S. Dreiding, *Helv. Chim. Acta* **46**, 1745 (1963).

here by formula I; isobetanidin is its 15-epimer[11] (III) and betanin its O-5-β-D-glucoside (II).[12,13] The first indication of the novel ring system[14] in betacyanins resulted from the structure analysis of a group of primary conversion products of betanidin, which we called neobetanidin derivatives.[1] It is now known[1] that these substances have the 14,15-dehydrobetanidin structure. In this paper we describe the details of these primary conversions.



I, R = H, betanidin
II, R = β-D-glucosyl, betanin



III, R = H, isobetanidin
IV, R = β-D-glucosyl, isobetanin

Conversions of betanidin to neobetanidin derivatives

In the treatment of a methanolic solution of betanidin or isobetanidin hydrochloride (V, $C_{18}H_{17}O_8N_2Cl$)[15–17] with a large excess of diazomethane, di-O-methyl-neobetanidin trimethyl ester (VII) was obtained as yellow crystals, m.p. 255°, $C_{23}H_{24}O_8N_2$, in good yield. The major absorption max of VII (403 nm, $\varepsilon = 32500$) was shifted in the presence of acid to 513 nm, $\varepsilon = 44500$, producing a deep violet color. This suggested that the chromophore of protonated VII was not significantly different from that of V. The introduction of five Me groups into betanidin was evident from the NMR spectrum of VII in $CDCl_3$ (Table 1), which displayed signals for five MeO groups: One six-proton singlet at 4·02[18] and three three-proton singlets at 3·97, 3·83 and 3·79. The parent peak (456)[19] in the mass spectrum[20] of VII confirmed the elementary analysis.

Another well characterized neobetanidin derivative was obtained by acetylation from betanidin hydrochloride trimethyl ester (VI): Although VI was available only

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[11] M. E. Wilcox, H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **48**, 1134 (1965).

[12] M. Piatelli, L. Minale and G. Prota, *Ann. Chim.* **54**, 955 (1964).

[13] M. E. Wilcox, H. Wyler, T. J. Mabry and A. S. Dreiding, *Helv. Chim. Acta* **48**, 252 (1965).

[14] This observation confirmed finally that the betacyanins were not structurally related to the flavylium salts, as implied by the early name "nitrogenous anthocyanin".

[15] H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **42**, 1699 (1959).

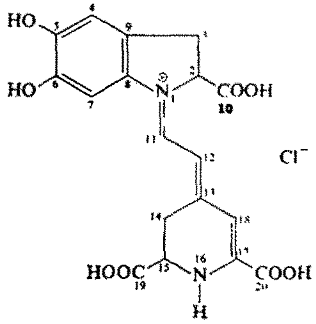
[16] The empirical formula given in Ref. 15 ($C_{19}H_{19-21}O_8N_2Cl$) was later modified to $C_{18}H_{17}O_8N_2Cl$ (see also Refs 1, 10 and 17).

[17] O. Th. Schmidt, P. Becher and M. Hübner, *Chem. Ber.* **93**, 1296 (1960).

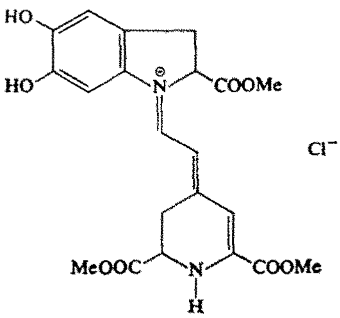
[18] All chemical shift values are reported in ppm, δ-scale.

[19] The M-2 peak (454) was actually more intense than the parent peak, suggesting a ready dehydrogenation of the dihydroindole ring.[20]

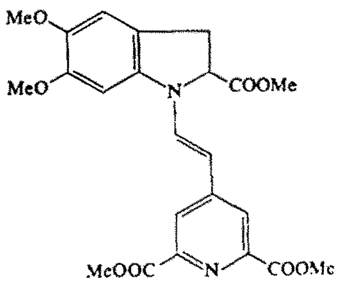
[20] We thank Dr. J. Seibl, Laboratory of Organic Chemistry, ETH, for the mass spectral data and their interpretation.



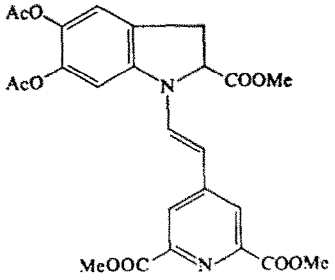
V



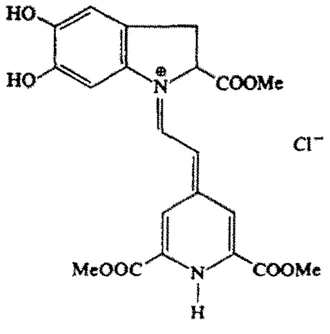
VI



VII



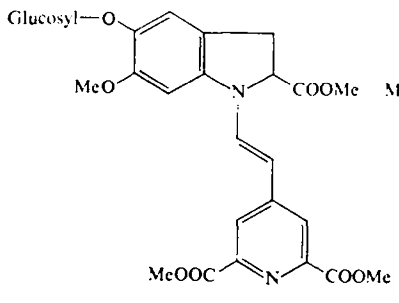
VIII



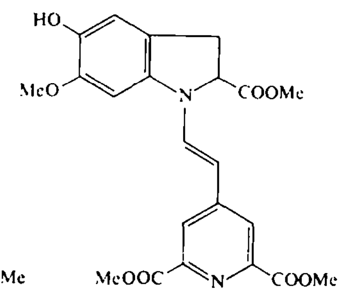
IX

in a crude form[21] from the acid catalyzed esterification of betanidin hydrochloride (V), its structure was confirmed by its NMR spectrum (all signals except the ones due to the MeO groups were very similar to those of I; see Experimental), by the analytical manifestation of approximately three MeO groups and by its acid hydrolysis back to a mixture of betanidin and isobetanidin (V).

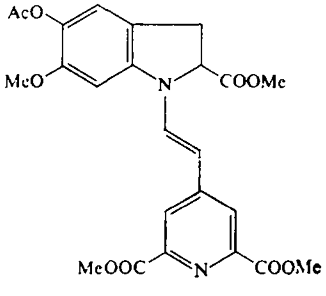
[21] This trimethyl ester (VI) has not been isolated in crystalline form and it is not known to what extent epimerization at C15 may have taken place during the methanolysis. No attempt was made to separate the C15-epimers of VI, since the asymmetry of this center was destroyed anyway in the conversion of VI to VIII.



X



XI



XII

In acetic anhydride and pyridine, VI yielded the crystalline 5,6-di-O-acetylneobetanidin trimethyl ester (VIII), mp. 200·5°, $C_{25}H_{24}O_{10}N_2$. Its absorption max in MeOH at 383 nm ($\varepsilon = 32500$) was shifted to 477 nm by the addition of acid. The presence, as indicated in the NMR spectrum of VIII (Table 1), of two phenolic acetate groups (2 three-proton singlets at 2·28 and 2·32)[18] and 3 MeO groups (a six-proton singlet at 4·03 and a three-proton singlet at 3·81) was the first definitive evidence that the eight oxygens of neobetanidin (and thus probably also of betanidin) corresponded to two phenolic OH and three carboxyl groups. Deacetylation occurred when VIII was allowed to stand in the acidic methanol solution for 6 hr leading to neobetanidin trimethyl ester hydrochloride (IX), which had an absorption max in 0·1N methanolic HCl at 520 nm. Compound IX (after deprotonation) could be reacetylated to VIII or methylated with diazomethane to VII.

Neobetanidin trimethyl ester hydrochloride (IX) was obtained directly from betanidin hydrochloride trimethyl ester (VI), when the latter was deprotonated in a methanolic solution by the addition of such bases as dimethylamine, pyridine or sodium acetate.

Betanin (II) was also converted to a neo-compound during methylation with diazomethane. The initially formed impure 6-O-methyl-neobetanin-trimethylester (X) was hydrolyzed with acidic methanol to 6-O-methyl-neobetanidin trimethyl ester (XI), which was characterized as the 5-O-acetyl derivative (XII)[22] and by its conversion to VII with diazomethane.

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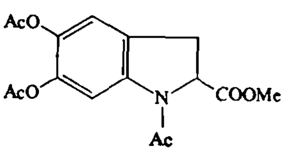
[22] These compounds (X, XI and XII) were used to determine the position of the glucose in betanin and the experimental details are recorded there.[18]

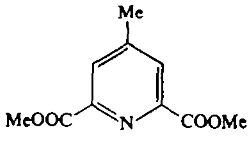
The overall reaction in the betanidin-to-neobetanidin conversions is a two electron oxidation, the mechanism of which is not yet understood. The transformation seems to be successful only under conditions where the betanidin trimethyl ester cation could be expected to be deprotonated; in an *acidic* medium, on the other hand, acetylation does not bring about conversion to the neo-series.[10] Since the yields in at least some of the conversions definitely exclude an intermolecular disproportionation of the dihydropyridine systems, molecular oxygen is suspected to be the oxidizing agent, although this has not yet been confirmed experimentally.

The structure of neobetanidin

Structures which account for all the properties of the neobetanidin substances and for their facile preparation from betanidin and betanin were deduced primarily from their NMR spectra. The interpretation required comparison with a number of model compounds including N-acetyl-5,6-diacetoxy-2,3-dihydroindole-2-carboxylic acid methyl ester (XIII) and 4-methylpyridine-2,6-dicarboxylic acid methyl ester (XIV).



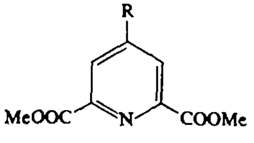
XIII



XIV

The latter two compounds had previously been derived from an alkaline degradation of betanidin and their structures established by comparison with authentic samples.[15,23] Their NMR spectra contained signals analogous to all those found in the spectrum of di-O-acetyl-neobetanidin trimethyl ester (VIII)[24] except two one-proton-doublets, which will be discussed later.

The presence of partial structure A in VIII was evident from the NMR comparison with XIV, since both VIII and XIV (Table 1) showed identical signals for the two carbomethoxyl groups (six-proton singlet at 4·03) and for the two β-hydrogens on the pyridine ring (two-proton-singlet at 8·10).[25]



A

From a NMR comparison with XIII it was evident that VIII also contained the partial structure B. Identical signals for the two acetyl groups (2·28 and 2·32 for both VIII and XIII) and very similar signals for the carbomethoxyl group (3·81 for VIII and 3·79 for XIII) were observed in the two compounds. Each displayed two one-proton-singlets in the region of aromatic protons (6·86 and 6·98 for VIII and 7·05 and

[23] H. Wyler and A. S. Dreiding, *Helv. Chim. Acta* **45**, 638 (1962).

[24] A traceout of the NMR spectrum of VIII can be found in the preliminary publication.[1]

[25] The fact that these pyridine hydrogens give a singlet signal indicates, that the rotation around the 12—13 bond must be fast despite the conjugation.

TABLE 1. NMR SIGNALS OF NEOBETANIDIN DERIVATIVES AND DEGRADATION PRODUCTS[a]

Compound Neobetanidin derivs.	Solvent		Aliphatic		Aromatic[b]		Vinyl		Pyridine	Substituents on the benzene ring	Ester methoxyls		
		H2	H3-*trans*	H3-*cis*	H4	H7	H11	H12	H14 H18	at C5 and C6	at C10	at C19 and C20	Miscellaneous
VII	$CDCl_3$	4·80 q (10 and 5)	3·14 q (17 and 5)	3·63 q (17 and 10)	6·63 s	6·78 s	7·71 d (14)	5·37 d (14)	8·10 s	methoxyls 3·97 s and 3·83 s	3·79 s	4·02 s	
VIII	$CDCl_3$	4·84 q (10 and 5)	3·20 q (17 and 5)	3·65 q (17 and 10)	6·86 s	6·98 s	7·68 d (14)	5·46 d (14)	8·10 s	acetates 2·28 s and 2·32 s	3·81 s	4·03 s	
XI	$CDCl_3$	4·78 q (10 and 5)	3·09 q (17 and 5)	3·59 q (17 and 10)	6·60 s	6·77 s	7·72 d (14)	5·33 d (14)	8·07 s	methoxyl at C6: 3·98 s	3·80 s	4·03 s	
XII	$CDCl_3$	4·80 q (10 and 5)	3·09 q (17 and 5)	3·59 q (17 and 10)	6·63 s	6·82 s	7·73 d (14)	5·42 d (14)	8·11 s	methoxyl: 3·89 s acetate: 2·32 s	3·80 s	4·03 s	
protonated													
VII[c]	CF_3COOH	~5·3 bm	3·3–4·1 bm		7·01 s	7·20 s	8·68 d (14)	5·86 d (14)	8·17 bs	methoxyl: 4·00 s and ~4·20 s	4·00 s	~4·20 s	
VIII	CF_3COOH	5·34 q (10 and 5)	3·46 bm	3·96 bm	7·25 s	7·52 s	8·70 d (14)	5·97 d (14)	8·39 s	acetates: 2·47 s and 2·50 s	4·02 s	4·25 s	
degradation products[d]													
XIII	$CDCl_3$	4·93 bm	3·10 bm	3·58 bm	7·05 s	8·14 s				O-acetates: 2·28 s and 2·32 s	3·79 s		N-acetate: 2·13 s
XIV	$CDCl_3$								8·11 s			4·03 s	C4-methyl: 2·57 s

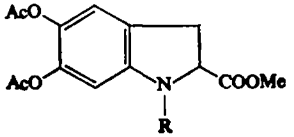
[a] For the symbols and units of measure used in this Table and for other information see introductory paragraph of the Experimental. All signals correspond in intensity to the number of protons indicated by the column heading. The figures in parentheses are coupling constants in c/s.

[b] A comment regarding the assignment of the aromatic proton signals is given in Footnote 27.

[c] These values are taken from a 100 mc spectrum.

[d] For the degradation products XIII and XIV the protons are numbered in this Table as if they were part of the neobetanidin system.

8·14 for XIII)[26,27] and an ABX signal pattern for the single proton at C2 and the two protons at C3 (Table 1).

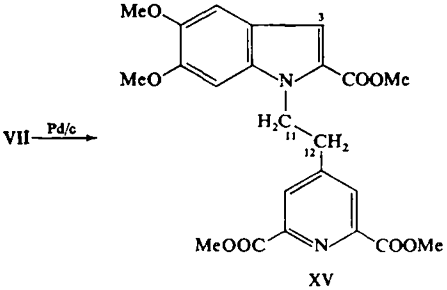


B

The NMR spectrum of VIII, however, does not contain signals corresponding to the N-acetyl group of XIII (2·13) nor one corresponding to the γ-Me group of XIV (2·57). These substituents have accordingly been replaced by R in the partial formulae A and B. Without the R's, A and B account for 23 of the 25 carbons of VIII. It is reasonable to conclude that the R's should be replaced by a connecting link consisting of the remaining two carbons in form of a —CH=CH— bridge. The two vinyl hydrogens account for the above mentioned two NMR doublets (AX-system, J = 14 c/s) at 7·68 and 5·46 and the large coupling constant points to a trans-configuration of the C11–C12 double bond. By connecting the ring systems A and B in this manner (formula VIII) the two nitrogens can be incorporated in a reasonable chromophoric system, which will be discussed later (see below). From the NMR spectra (Table 1) it is clear that the above mentioned compounds VII, XI and XII also belong to the same structure type (neobetanidin)[28] as VIII.

A disproportionation reaction

The presence of the connecting vinylene group (C11 and C12) was confirmed by a palladium catalyzed disproportionation reaction, which converted the yellow di-O-methyl-neobetanidin trimethylester (VII) to the colorless di-O-methyl-2,3-dehydro-11, 12-dihydro-neobetanidin trimethyl ester (XV), m.p. 178°.



XV

The reaction consisted of an aromatization of the dihydroindole system at the expense of hydrogenation of the vinylenebridge. These changes expressed themselves

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[26] The lowfield signal (8·14) in XIII can be attributed to H7, which is deshielded by the neighboring N-acetyl group. The preferred conformer of XIII has the amide oxygen directed towards H7. Additional evidence regarding this point will be published later.

[27] In the case of betanidin I (evidence to be published later) the lowfield aromatic signal could be assigned to H7. On the basis of the assumption that the similarities of structure are sufficient a corresponding assignment in the neobetanidin derivs may be made.

[28] The parent compound of this series, neobetanidin itself has not been isolated so far.

in the NMR spectrum of the product (XV), where the ABX- and AX-systems of VII had disappeared and were replaced by a lone indole-proton- and by two neighbouring methylene signals (Table 2). The mass spectrum confirmed the mol. wt. of XV (456) and the UV absorption max (320 nm, insensitive to the addition of acid) showed that the chromophoric system characteristic for the neobetanidin compounds had been destroyed.

Table 2. Changes in NMR-signals on palladium catalyzed disproportionation of indolin-enamines[a]

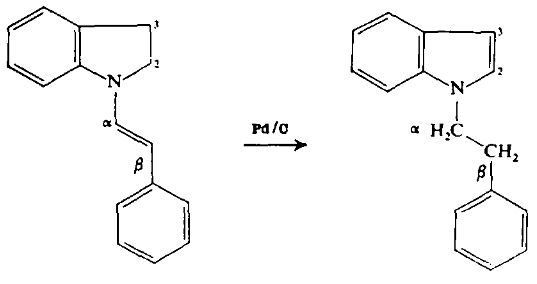
	H2	H3		H11	H12
VII	one proton 4·80 q (10 and 5)	two protons 3·14 q (17 and 5)	 3·63 q (17 and 10)	one proton 7·71 d (14)	one porton 5·37 d (14)
XV	—	one proton 6·53 s		two protons 4·77 t (7)	two protons 3·21 t (7)

	H2	H3	Hα	Hβ
XVI	two protons 3·76 t (8)	two protons 2·98 t (8)	one proton[b] 7·25 d (14)	one proton 5·36 d (14)
XVII	one proton 6·72 d (3·3)	one proton 6·28 d (3·3)	two protons 4·24 t (7)	two protons 2·98 t (7)

[a] The symbols and the units of measure used in this Table are explained in the introductory paragraph of the Experimental.

[b] Half of this signal was hidden under the aromatic proton multiplet in the 60 mc spectrum, but could be identified in a 100 mc spectrum and with the aid of spin decoupling.

As a model compound for comparison, N-styrylindolin (XVI), m.p. 81°, was synthesized from indolin and phenylacetaldehyde. Its NMR spectrum (CCl_4) showed the signals typical for the enaminic vinyl-hydrogens as well as for the indolin-methylene protons (Table 2). An analogous palladium disproportionation converted XVI to the oily N-(β-phenylethyl) indole (XVII), the structure of which was confirmed by its NMR spectrum (Table 2 and Experimental).

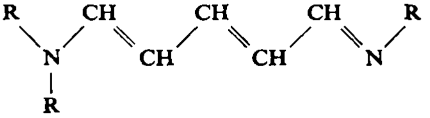


XVI XVII

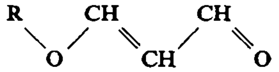
The 1,7-*diazaheptamethin chromophore in neobetanidin*

The neobetanidin derivatives belong to a group of compounds for which König proposed the expression "polymethin-pigments."[29–31]

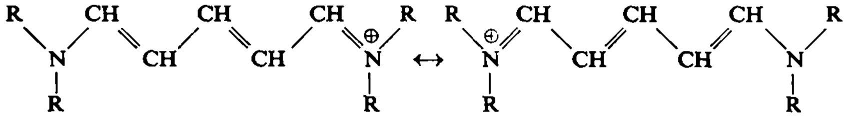
For the discussion of the *chromophore* we have found it useful to introduce a simplifying modification of the excellent and very broadly applicable nomenclature of König[30,31] in such a way as to *include in the count of methin units also the terminal atoms of the conjugated systems. Any heteroatoms at the ends of, or within, the chains are named and numbered by the "replacement name" rule.*[32,33] In this way the structure of the chromophore and its substituents can be recognized directly in the name.[34] Hydrogens are not mentioned and the numbering starts with the first doubly bound methin in the simplest valence bond formula. As an illustration, two uncharged and two charged chromophoric systems are displayed here:



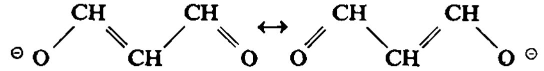
1,7,7-*Trisubstituted*-1,7-*diazaheptamethin system*



5-*Substituted*-1,5-*dioxapentamethin system*



1,1,7,7-*Tetrasubstituted*-1,7-*diazaheptamethonium system*



1,5-*Dioxapentamethide system*

This nomenclature can readily be extended to more complicated systems (for instance, as discussed by König[30]) or to the chromophores of cyanine and other dyes.

Neobetanidin derivatives can now be said to contain a 1,7-diazaheptamethin system, in which the first four methin units are a part of a pyridine ring.[35] The auxochromic substituents are a carbomethoxyl group at position 2 (formula XVIII) and an alkyl- as well as a 4-catechyl group at position 7. The latter group must

[29] W. König, *Chem. Ber.* **55**, 3297 (1922).

[30] W. König, *J. Prakt. Chem.* [2] **112**, 1 (1926).

[31] H. A. Staab, *Einführung in die theoretische organische Chemie* p. 323 ff. Verlag Chemie, Weinheim (1959).

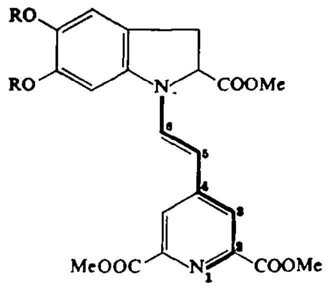
[32] IUPAC 1957 Rules, *J. Am. Chem. Soc.* **82**, 5545 (1960).

[33] This procedure was first proposed by Dr. Harry Dugger, while working in this laboratory. It has already been applied in some of our previous publications, see e.g. H. Wyler, M. E. Wilcox and A. S. Dreiding, *Helv. Chim. Acta* **48**, 361 (1965), Footnote 4.

[34] König's distinction between "mesochrome" and "perichrome" now becomes unnecessary. The difference in the treatment of heteroatoms at the ends and in the middle of the chain is also avoided.

[35] According to Hamer,[36] the neobetanidin derivs are still not cyanine dyes, in which both nitrogens must be surrounded by three carbon-neighbours.

[36] F. M. Hamer *The Cyanine Dyes and Related Compounds* Monograph in the series *The Chemistry of Heterocyclic Compounds* (Edited by A. Weissberger) Interscience, New York (1964).



XVIII

TABLE 3. LONGWAVE ABSORPTION BANDS IN THE ELECTRON SPECTRUM AND pK_a* VALUES OF SOME NEOBETANIDIN DERIVATIVES

1	2	3	4	5
Compound	Absorption maximum[a] nm		Shift of maximum on protonation nm	pK_a*
	in CH_3OH	in CH_3OH/H^+		
VIII	383	477	94	1·6
IX	410[b]	520	110	2·4
VII	403	513	110	2·0
XI	407	519	112	
XII	393	496	103	1·7

[a] The ε-values range between 30000 and 45000.

[b] In this case deprotonation of IX occurred spontaneously to a large extent in the dilute spectroscopic soln.

exert a strong influence on the yellow chromophore since acetylation of one or both of the catechol oxygens produces a hypsochromic shift of 14 and of 27 nm (compare XI with XII and deprotonated IX with VIII in column 2 of Table 3).

The basic 1,7-diazaheptamethin system in neobetanidin derivatives can be protonated to give a 1,7-diazaheptamethonium chromophore with a bathochromic shift of the major absorption maximum in the order of 100 nm (see column 4 of Table 3). This shift, earlier known as "halochromism", reflects a certain equalization of carbon-carbon bond lengths along the π-electron chain when the system is charged.[31,37] A qualitatively, but not quantitatively, similar effect had been observed due to protonation[38] and due to quaternization[39] of several "anhydro bases" of cyanine systems. The large bathochromic shift on protonation permits a spectroscopic estimation of pK_a* values, which were determined for VII, VIII, IX and XII (Table 3, column 5). The weakly basic character of these neobetanidin esters[40] is probably due to the fact

[37] A. Maccoll, *Quart. Rev.* **1**, 16 (1947); J. R. Platt, *Handbuch der Physik* Band 37/2, *Moleküle* II (Edited by S. Flügge) Springer Verlag (1961).

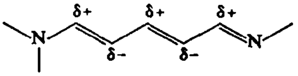
[38] Ref. 36, page 700. A. I. Kiprianov and V. A. Shrubovich, *J. Gen. Chem. USSR* **26**, 2891, English Translation 3215 (1956). L. G. S. Brooker, F. L. White, G. H. Keyes, C. P. Smyth and P. F. Oester, *J. Am. Chem. Soc.* **63**, 3192 (1941).

[39] Ref. 36, page 701. L. G. S. Brooker, R. H. Sprague, C. P. Smyth and G. L. Lewis, *J. Am. Chem. Soc.* **62**, 1116 (1940).

[40] Betanidin derivs have a much higher pK_a. This will be described in another publication.

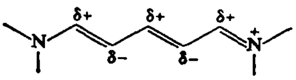
that a part of the 1,7-diazaheptamethin system is incorporated in a pyridine ring which, moreover, has two carbomethoxyl groups in the α-positions.

We now turn to a consideration of the NMR signals due to the vinyl-hydrogens (H11 and H12) in neobetanidin derivatives, which were mentioned already above. The large difference in chemical shift (Δδ—values for VII 2·36, for VIII 2·22, for XI 2·39 and for XII 2·31; see column under the heading "vinyl" in Table 1) seems to be a general property of 1,ω-diazapolymethin systems and can be accounted for by an alternatingly low or high electrondensity on the methin groups as shown in the extreme valence bond formula XIX



XIX

In the NMR spectra of the *protonated* neobetanidin derivatives (in CF_3COOH) the vinyl hydrogen signals are separated even more widely (for protonated VIII by 2·73 and for protonated VII by 2·75 ppm, see "vinyl" column in Table 1). This effect has been demonstrated and rationalized for a number of linear 1,ω-diazapolymethinium systems (general valence bond formula XX) by Scheibe *et al.*[41] and



XX

also by Dauben.[42,43] On this basis a definite assignment can be made for the lowfield AX-doublet in the neobetanidin NMR-spectra as belonging to H11 and the highfield doublet to H12.

Acknowledgement—This work was supported in part by the *Schweizerische Nationalfonds zur Förderung der Wissenschaftlichen Forschung and in part by F. Hoffmann-La Roche & Co. AG. Basel.* We are also grateful to the *Jubiläumsspende für die Universität Zürich* for the acquisition of certain equipment and to the *American Swiss Foundation for Scientific Exchange* for a travel grant to T. J. Mabry.

EXPERIMENTAL

The m.ps are reported uncorrected. For column chromatography a neutral Woelm alumina was stirred with dil HCl for several hr, washed to neutrality by repeated decantation with distilled water and dried at 105° for 3–4 hr. Just before use this material was deactivated by adding 3% water to give activity 2. The UV spectra were obtained with a Beckman model DK2, the IR spectra in a Perkin-Elmer model 21 and the NMR spectra with Varian A-60 and HR-60 model spectrometers. The spectra are recorded here as follows: UV[44] (solvent): max wavelength (intensity) nm (ε); IR (solvent): wavelength (intensity symbol, st, m, w) μ; and NMR (solvent): δ-value in ppm/multiplicity (J-values in c/s) number of protons pr (assignment), ppm (c/s). The IR spectra are not described in

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[41] G. Scheibe, W. Seiffert, H. Wengenmayr and C. Jutz, *Ber. Bunsenges. physik. Chem.* **67**, 560 (1963).

[42] H. Dauben, private communication: G. Feniak and R. B. Lund, both dissertations, University of Washington.

[43] J. Parikh in this laboratory synthesized a few new compounds of this type and confirmed the generality of the effect.

[44] The symbol UV is used in this Experimental to mean electronic spectra, both in the UV and in the visible region.

all details; in most cases only the region between 5·5 and 7·0 μ is reported. The assignments of the NMR signals are indicated by labelling the hydrogens or H-containing substituents according to the numbers of the C atoms (see formula V) to which they are attached. The following abbreviations are used in the NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, sm = multiplet with fine splitting. The letter b before any of these symbols indicates that the signal is broad. TMS was used as internal standard. Early NMR spectra with the HR-60 were measured at Varian AG, Zurich, Switzerland. The elementary analyses were performed at our institute's microlaboratory by Mr. H. Frohofer and his staff.

5,6-*Di*-O-*methyl-neobetanidin trimethyl ester* (VII)

Betanidin hydrochloride or a mixture of betanidin and isobetanidin hydrochloride V (100 mg, UV max 542 nm in 0·1N methanolic HCl, ε 41500; pure betanidin has an ε of 49400)[18] was dissolved in 50 ml dry MeOH. The diazomethane produced by the standard procedure[45] from 7 g of diazald (*p*-toluene-N-dimethylsulfonamide) was distilled as an ether-CH_2N_2 soln into the betanidin hydrochloride soln which was chilled to 0°. The violet color of betanidin disappeared during the distillation. The mixture was allowed to stand overnight at 0–10°. The residue obtained on removing the solvent was chromatographed with CH_2Cl_2 over 3 g of acid-washed activity-2 alumina. A yellow band was eluted from the column with CH_2Cl_2. On evaporation of the solvent (71 mg) and recrystallization from MeOH containing a little CH_2Cl_2, 61 mg (67% yield) of 5,6-*di*-O-*methyl-neobetanidin trimethyl ester* (VII) was obtained as two crops of bright yellow needles; m.p. 254–255°; $[\alpha]_D^{23}$ −181° (*c* 2·19 in CH_2Cl_2); UV (MeOH): max 403 (32500), 317 (6500), 267 (12500) and 224 (13500) nm (ε). IR (CH_2Cl_2): 5·79 (st), 5·84 (st), 6·14 (st), 6·33 (st), 6·65 (st), 6·97 (st) μ. In 0·1N methanolic HCl, the protonated species of VII had max 513 (44500), 315 (broad, 5300), 275 (shoulder, 11500), and 258 (13000) nm (ε). The NMR spectrum in $CDCl_3$ is summarized in Table 1. The NMR spectrum of a freshly prepared solution of VII in CF_3COOH (violet, as VII is protonated) at first exhibited only diffuse signals. These became readably clear (but not sharp) after the soln had stood at room temp over night: NMR (CF_3COOH): 8·68/bd (J = 14), 1pr (H11); 8·17/bs, 2pr (H14, H18); 7·20/bs, 1pr (H7); 7·01/bs, 1pr (H4); 5·86/bd (J = 14), 1pr (H12); 5·3/bm, 1pr (H2); 4·1–3·3/bm (not integratable, 2 × H3); δ (c/s); in the region of 4·20 to 3·95 there are 4 singlets which are attributed to the five MeO groups; their intensities differ in such a way that a clear interpretation is not evident. This, as well as the broadness of the other signals, may be due to a restricted rotation around the 12–13 bond in the more conjugated system of *protonated* VII. (Found: C, 60·61; H, 5·38; N, 6·08; MeO, 33·92; mol. wt. (mass spec.), 456. $C_{23}H_{24}O_8N_2$ requires: C, 60·52; H, 5·30; N, 6·14; MeO, 34·00%; mol. wt., 456·44.)

Betanidin hydrochloride trimethyl ester VI

A soln of 100 mg of a mixture of betanidin- and isobetanidin hydrochloride (77% spectral purity, based on ε = 49400 for the pure aglucone[18]) in 30 ml of dry HCl (1N) in MeOH was allowed to stand for 16 hr at 50° in an atm of N. After removing the volatile components at reduced press, the residue (only partially crystalline) was dried in a high vacuum for 45 hr over P_2O_5 and KOH. This crude sample of betanidin-isobetanidin hydrochloride trimethyl ester[21] (VI) showed UV (0·1N methanolic HCl): max 550 (35500), 323 (shoulder, 13000), 301 (16000), 276 (15000) nm (ε); UV (MeOH): max 553 (36500) nm (ε); UV (H_2O): max 542 (36000) nm (ε); NMR (CF_3COOH): 8·60/d (J = 13), 1pr (H11); 7·31/s, 1pr (H7); 7·01/s, 1pr (H4); 6·55/s, 1pr (H18); 6·24/d (J = 13), 1pr (H12); 5·47/bm, 1pr (H2); 4·60/bm, 1pr (H15); 4·08/s, 3pr (MeO at C20); 3·97/s, 6pr (2 × MeO at C19 and C10); 3·57/bm, 4pr (2 × H3, 2 × H14) δ(c/s). (Found: C, 54·84; H, 5·18; N, 5·76; Cl, 8·21; MeO, 17·95. $C_{21}H_{23}O_8N_2$ requires: C, 54·02; H, 4·97; N, 6·00; Cl, 7·60; MeO, 19·94%.) Several attempts to crystallize this compound were unsuccessful.

5,6-*Di*-O-*acetyl-neobetanidin trimethyl ester* (VIII)

Crude betanidin-isobetanidin hydrochloride trimethyl ester, prepared from 200 mg of 77% pure betanidin-isobetanidin hydrochloride as described above, was treated with 15 ml Ac_2O and 0·5 ml pyridine for 6 hr at 40° without exclusion of O. After removing the reagents at reduced press, the residue was chromatographed over 6 g of acid-washed activity-2 alumina. Elution of the yellow band

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[45] Th. J. de Boer and H. J. Backer, *Org. Syn.* **36**, 16 (1956).

with CH_2Cl_2 and recrystallization of the residue from CH_2Cl_2–MeOH afforded 90 mg (49% yield, based on betanidin entered into the reaction) of *5,6-di-O-acetyl-neobetanidin trimethyl ester* (VIII) as bright yellow needles, m.p. 200·5° in two crops; $[\alpha]_D^{24}$ −151°, (*c* 2·94, Chf). UV (MeOH): max 383 (32500), 313 (7700), 265 (16000) and 222 (shoulder, 22500) nm (ε); UV (0·1N methanolic HCl): max 477 (41800), 310 (4000) and 268 (15000) nm (ε). On standing, this acidic soln displayed a slow change of the max from 477 over 504 to 523 nm, which is due to the methanolysis of the acetyl groups. IR (KBr): 5·68 (st), 5·75 (st), 5·81 (st), 5·91 (m), 6·13 (st), 6·32 (st), 6·66 (st), 6·96 (st) μ. The NMR spectrum is described in Table 1. (Found: C, 58·65; H, 5·00; N, 5·56; MeO, 17·82; MeCO, 18·22; C-Me, 5·72. $C_{25}H_{24}O_{10}N_2$ requires: C, 58·95; H, 4·72; N, 5·47; MeO, 18·17; MeCO, 16·80; C-Me, 5·87%.) The high acetyl value found for VIII may be accounted for by the formation of formic acid from a skeletal degradation during the analytical procedure. This was confirmed by the observation that even unacetylated betanidin and neobetanidin derivatives produced a small amount of volatile acid.[23]

Neobetanidin trimethyl ester hydrochloride (IX)

A. *Preparation by methanolysis of* VIII. 40 mg of 5,6-di-O-acetyl-neobetanidin trimethyl ester (m.p. 200·5°) were allowed to stand in 3 ml of 1N methanolic HCl at room temp in the absence of O. After 1 hr, the soln was violet and overnight a voluminous ppt had formed which appeared to be a highly solvated violet-colored crystalline compound. After filtering and drying this material under high vacuum, the yield was 26 mg (74%) of *neobetanidin trimethyl ester hydrochloride* (IX), m.p. 254°. Evaporation of the mother liquor to dryness yielded an additional 9 mg (total yield 99%). UV (0·1N methanolic HCl): max 520–523 (49000), 300–320 (broad, 8500), 280 (shoulder, 9700), 258 (13300) nm (ε). (The intensities may be a little low since drying was difficult due to the hydroscopic nature of the substance). Dissolving this hydrochloride (IX) in spectroscopic concentrations in MeOH resulted in almost complete deprotonation, so that the addition of only a small amount of pyridine vapor allowed the measurement of the absorption spectrum of the free base; UV (MeOH): max 410 (28000) nm (ε). This spectroscopic soln is stable for at least 120 hr. In a more basic soln the substance decomposed more rapidly (a few min if the soln was strongly basic). The extent of the decomposition could be determined by reacidification with 0·1N methanolic HCl and measuring how much of the violet color of neobetanidin trimethyl ester hydrochloride was regenerated. IR (KBr): 3–3·5 (st, broad), 5·79 (st) with shoulders at 5·84 and 5·89, 6·37 (st, broad), 6·62 (w), 6·96 (m) μ. (Found: C, 54·20; H, 5·1; N, 5·99; MeO, 19·48; MeCO, 0·90; Cl, 7·87. $C_{21}H_{20}O_8N_2 \cdot HCl$ requires: C, 54·25; H, 4·56; N, 6·03; MeO, 20·03; MeCO, O; Cl, 7·63%.) The small amount of acetyl found may be accounted for as pointed out above in connection with the analysis of VIII.

B. *Preparation directly from betanidin hydrochloride trimethyl ester* (VI). 2 ml of a degassed mixture of 33% dimethylamine in MeOH was distilled to a dried film of betanidin hydrochloride trimethyl ester (crude, prepared as described above from 30 mg of a betanidin isobetanidin hydrochloride mixture, V) in an evacuated (while frozen) and closed system. The violet color changed to yellow while the pigment dissolved quickly in the basic distillate. After 30 min, this soln was concentrated to dryness and the residue acidified to give a deeply violet solution by distilling an excess 1N methanolic HCl onto it. These operations were also performed at the vacuum line. The product was identified as neobetanidin trimethyl ester hydrochloride (IX) by its absorption max at 520 nm in acid soln and at 410 nm after addition of some base. The absorption intensities compared with that of the educt (VI) indicated an approximate yield of 80%. It appears as if the oxygen had not been completely excluded from this reaction system. If the same procedure was performed in the open atmosphere no violet color was formed after the reacidification. This is in accord with experience described above under A that the free neobetanidin-trimethylester (deprotonated IX) is highly sensitive to oxygen.

Identical conditions at the vacuum line, but using pyridine or AcONa as the base, also produced neobetanidin trimethyl ester hydrochloride (IX). In the latter case the AcONa (200 mg) was added as a solid to the dry film of educt VI (from 90 mg of V) and MeOH was distilled to the mixture.

Acetylation and methylation of neobetanidin trimethyl ester hydrochloride (IX)

The dry violet residue from the acid methanolysis of 30 mg of VIII, (described above) was treated with an excess of Ac_2O and pyridine. After evaporation and chromatography (see above) it was possible to isolate VIII, m.p. 200°, identified by its IR spectrum.

A mixture of diazomethane and ether was distilled to the same residue from the acid methanolysis of 30 mg of VIII. Concentration, chromatography and crystallization (see above) yielded 20 mg of VII as yellow needles, m.p. 254°. The UV and NMR spectra were identical with those of the material prepared directly from V.

Determination of the pK_a *values of the neobetanidin derivatives* VII, VIII, IX and XII

A given neobetanidin derivative exhibits a strong absorption max near 400 nm (max 1) as the free base (B) and one near 500 nm (max 2) as the conjugate acid (HB⁺); the exact wavelengths $\lambda_{\max 1}$ and $\lambda_{\max 2}$ depend on the substituents attached to the phenolic oxygens (Table 3). At certain pH's both species (B and HB⁺) must be present, so that a pK_a-value for a specific compound can be calculated from

$$pK_a = \log\frac{[HB^+]}{[B]} + pH$$

The concentrations of the free base [B] and of the protonated form [HB⁺] are derivable from the intensities of the two absorption maxima at this pH (E_1^{pH} and E_2^{pH}), if the absorption intensities of the pure base (E°_{1B}) and of the pure protonated species ($E^\circ_{2HB^+}$) at their respective maxima (max 1 and max 2) were known.

$$pK_a = \log\frac{E_2^{pH}/E^\circ_{2HB^+}}{E_1^{pH}/E^\circ_{1B}} + pH$$

Since B can also absorb some light (E°_{2B}) at the wavelength of max 2 and, conversely, HB⁺ absorbs also slightly ($E^\circ_{1HB^+}$) at the wavelength of max 1, the formula must be corrected to

$$pK_a = \log\frac{(E^\circ_{2B})(E_1^{pH}) - (E^\circ_{1B})(E_2^{pH})}{(E^\circ_{1HB^+})(E_2^{pH}) - (E^\circ_{2HB^+})(E_1^{pH})} + pH$$

If all measurements are made by equal dilutions from a stock solution of the compound the exact concentration need not be known and the observed optical densities (od) can replace the absorption intensities (E).

For the od^{pH}-measurements, a stock soln in MeOH was diluted 1:9 with suitable McIlvaine buffer mixtures and the apparent pH was determined by means of a glass electrode immediately after the optical evaluation. Both B and HB⁺ are not stable in these largely aqueous solns, so that useful intensity values had to be obtained by an extrapolation procedure. For this, the rates of decomposition were followed by monitoring separately the optical density at max 1 (od_1) and at max 2 (od_2) as a function of time. Extrapolation to zero time gave od_1^{pH} and od_2^{pH}. At the extremely high or low pH's in aqueous solns, at which only B or only HB⁺ would be present, the compounds were so unstable that this procedure was inconvenient. Thus the required standard values od°_{1B} and od°_{2B} were measured in methanol and $od^\circ_{2HB^+}$ and $od^\circ_{1HB^+}$ in methanolic HCl, in which the compounds were sufficiently stable.

Thus we define (at equal concentrations)

od_1^{pH}: Optical density of the buffered soln of B (known pH) at the absorption max near 400 nm (extrapolated from a decomposition curve to zero time).

od_2^{pH}: Optical density of the same buffered soln of B (known pH) at the absorption max near 500 nm (extrapolated from a decomposition curve to zero time).

od°_{1B}: Optical density of a soln of B in MeOH at the absorption max near 400 nm.

od°_{2B}: Residual optical density of the same soln of B in MeOH at the wavelength of the absorption max of HB⁺ near 500 nm.

$od^\circ_{2HB^+}$: Optical density of a soln of HB⁺ in 0·01N methanolic HCl at the absorption max near 500 nm.

$od^\circ_{1HB^+}$: Residual optical density of the same soln of HB⁺ in 0·01N methanolic HCl at the wavelength of the absorption max of B near 400 nm.

and use the approximate equation for pK_a^*:

$$pK_a^* = \log\frac{(od^\circ_{2B})(od_1^{pH}) - (od^\circ_{1B})(od_2^{pH})}{(od^\circ_{1HB^+})(od_2^{pH}) - (od^\circ_{2HB^+})(od_1^{pH})} + pH$$

In practice the following procedure was followed: From a stock soln of approximately 0·8 mg of the neobetanidin derivative (VII, VIII, IX or X) in 10·0 ml MeOH, 0·3 ml aliquots were taken and made up to 3·0 ml spectroscopic samples with (a) MeOH (b) approximately 0·01N methanolic HCl and (c) McIlvaine standard buffer solutions[46] of the appropriately chosen pH. In this way, all solns contained about 8 × 10^{-3} mg/ml. In MeOH (a) and in 0·01N methanolic HCl (b) the optical densities at max 1 and at max 2 remained constant over several hr,[47] so that values for od°_{1B}, $od^{\circ}_{2HB^+}$ and $od^{\circ}_{1HB^+}$ were obtained (od°_{2B} was zero in all the cases examined here). The measurements in the buffered solns were made within as short a time as possible (about 7–10 sec) after mixing the methanolic stock soln with the aqueous buffer. At this point, the recorder needle of the automatic spectrophotometer was set at $\lambda_{max\,1}$ and its progress was used as a measure of time. By extrapolating to time = 0 a value for od_1^{pH} was estimated. The same procedure was repeated with a new and freshly prepared soln of the same pH at $\lambda_{max\,2}$ to get a value for od_2^{pH}. Immediately after this measurement the apparent pH of the spectroscopic soln was checked with a Phillips pH-meter. The observed results and the calculated $pK_a{}^*$ values are summarized in Table 4.

The method employed here leads to approximate (therefore starred) values, which should be used for comparison only with similarly determined $pK_a{}^*$'s. The sources of deviation from real pK_a values are: (1) The od^{pH} were measured in about 10% MeOH aq, whereas the standard od° in MeOH. (2) The pH's were determined in 10% aqueous MeOH solns with a pH-meter calibrated against aqueous buffers. (3) The extrapolation procedure is only approximate, since the rapidity of the decomposition made it difficult to get good short time od-readings.

5,6-*Di-O-methyl-*2,3-*dehydro-*11,12-*dihydro-neobetanidin trimethyl ester* (XV)

A soln of 114 mg (0·25 mmole) VII in 25 ml xylene containing 1 ml tetralin was refluxed with 300 mg Pd/C (5% Pd) for 3 hr. The catalyst was filtered off from the warm soln and washed with hot xylene. The filtrate was removed under vacuum, leaving a solid residue which was taken up in ca. 5 ml Chf and filtered through 1 g aluminum oxide. The dried residue from the filtrate was crystallized from boiling MeOH yielding 51 mg (45%) of XV as colorless needles m.p. 177–178° (vac.). The compound can be distilled at 205–210°/0·01 Torr to give a colorless hard solid of the same m.p. UV (MeOH): max 320 (18600), 260 (6050) nm (ε); UV (0·1 methanolic HCl) as in MeOH. IR (KBr): very strong broad band, split at the very top into 3 humps (5·77, 5·83, 5·91), 6·11 (vw), 6·23 (m), 6·56 (m), 6·67 (m), 6·79 (m), 6·93 (st) μ. NMR ($CDCl_3$): 8·01/s, 2pr (H14, H18); 7·15/s, 1pr (H7)[27]; 6·95/s, 1pr (H4);[27] 6·53/s, 1pr (H3); 4·77/t (J = 7) 2pr (2 × H11); 3·21/t (J = 7), 2pr (2 × H12); 3·97/s, 6pr (C19 and C20 carbomethoxyl); 3·85/s, 9pr (C10-carbomethoxyl and methoxyls at C5 and C6) δ(c/s). The mass spectrum[30] shows a mol. wt. of 456, as calculated. (Found: C, 60·64; H, 5·54; N, 6·21; MeO, 34·30. $C_{23}H_{24}N_2O_8$ requires: C, 60·52; H, 5·30; N, 6·14; MeO, 34·00%.)

N-(*β-Styryl*) *indolin* (XVI)

Freshly distilled indolin (1·1 g; 10 mmole) in 10 ml dry benzene and 1·44 g (12 mmole) phenylacetaldehyde in 15 ml benzene were mixed with cooling. After refluxing for 2 hr, the solvent was distilled off under vacuum, leaving a yellow oily residue which solidified after standing at room temp for 2 hr. It was crystallized from ligroin to give 1·9 g (88%) N-(*β-Styryl*) *indolin* (XVI) as pale yellow needles, m.p. 81°. The product was sublimed at 120°/0·005 Torr to give a crystalline solid, which remained colorless for a short time in air. UV (MeOH): max 228 (16800), 305 (23100), 345 (31000) nm (ε); UV (0·1N methanolic HCl): max 302 (18900) nm (ε). IR (CH_2Cl_2): 6·10 (st), 6·22 (shoulder, st), 6·29 (st), 6·36 (w), 6·73 (st), 6·84 (w) μ. IR (KBr): 10·83 (m) μ. NMR (CCl_4): 7·25/d (J = 14, hidden partially under the aromatic protons), 1pr (H10); 6·86/m, 9pr (aromatic protons); 5·36/d (J = 14), 1pr (H11); 3·76/t (J = 8), 2pr (2 × H2); 2·98/t (J = 8), 2pr (2 × H3) δ(c/s). NMR (CF_3COOH): 0·93/txt (J = 6·3, J = 2·5), 1pr (Hα); 7·68/bs, 4pr (H-aromatic in indolin); 7·47/bs, 5pr (H-aromatic in styryl); 4·86/bt (J = 6·9, J = ~2·5, not clear), 2pr (2 × H2); 4·36/d (J = 6·3), 2pr (2 × Hβ); 3·64/t (J = 6·9), 2pr (2 × H3) δ(c/s). (Found: C, 86·53; H, 7·03; N, 6·41; $C_{16}H_{15}N$ requires: C, 86·84; H, 6·83; N, 6·33%.)

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[46] Various proportions of 0·1M citric acid and 0·2M Na_2HPO according to the *Handbook of Chemistry and Physics*.

[47] Methanolysis of the acetyl group in the methanolic HCl solns, as evidenced by a change of color from red to violet, was noticeable only after 6–8 hr.

TABLE 4. OPTICAL DENSITIES AND pH-VALUES USED FOR THE CALCULATION OF THE pK_a^* OF VII, VIII, IX AND XII

Cpd	λ_{max1} nm	λ_{max2} nm	od°-values				od^{pH}-values								Average pK_a^*
			in methanol		in 0·01 N methanolic HCl		In first buffer				In second buffer				
			od°_{1B}	od°_{2B}	$od^{\circ}_{2HB^+}$	$od^{\circ}_{1HB^+}$	at pH	od_1^{pH}	od_2^{pH}	pK_a^*	at pH	od_1^{pH}	od_2^{pH}	pK_a^*	
VII	403	513	0·60	0	0·77	0·05	2·20	0·33	0·28	2·03	2·00	0·31	0·34	1·96	2·00
XII	393	496	0·72	0	0·82	0·14	2·58	0·60	0·13	1·71	2·22	0·58	0·19	1·72	1·72
VIII	383	477	0·62	0	0·75	0·12	2·21	0·50	0·09	1·51	3·20	0·54	0·02	1·77	1·64
IX	410	520	0·68[a]	0	0·88	0·07	2·16	0·35	0·42	2·44	3·22	0·60	0·10	2·33	2·38

[a] Because cpd IX was dissolved as the hydrochloride a small amount of HB^+ remained in the methanolic soln. The value recorded here was measured after the addition of some pyridine vapors to the cuvette.

N-(*β-Phenylethyl*) *indole* (XVII)

Compound XVI (442 mg; 2 mmole) and 500 mg Pd/C (5% Pd) were heated in 25 ml refluxing xylene for 1·5 hr. After filtering off the catalyst, the solvent was removed under vacuum and the oily residue distilled at 95–100°/0·005 Torr in a kugelrohr to give 410 mg (92%) of XVII as colorless oil, which turned to yellowish brown in air after a short time. UV (MeOH): max 291 (7200), 274 (1340), 282 (1350), 293 (1050) nm (ϵ); UV (0·1N methanolic HCl): as in MeOH. IR (CH_2Cl_2): 6·22 (w), 6·60 (m), 6·72 (m), 6·83 (st) μ. NMR (CCl_4): 7·09/m, 9pr (aromatic protons); 6·72/d (J = 3·3), 1pr (H2); 6·28/d (J = 3·3), 1pr (H3); 4·24/t (J = 7), 2pr (H10); 2·98/t (J = 7), 2pr (H11) δ(c/s). (Found: C, 86·73; H, 6·91; N, 6·40. $C_{16}H_{15}N$ requires: C, 86·84; H, 6·83; N, 6·33%.)