at C(2) and C(4) should show an even larger $\Delta\nu_{NO}^{N}$ value due to the smaller distance between the proton and the centre of the N–O dipole. Therefore, from the observed $\Delta\nu_{NO}^{N}$ values in Table 2 one has to conclude that the first isomer (m.p. 252°; $H_{6\beta,7\beta}$: $\Delta\nu_{NO}^{N} = -101$ Hz; $H_{2a,4a}$:$\Delta\nu_{NO}^{N} = -74$ Hz) must be assigned structure 1 and the second isomer ($H_{2a,4a}$:$\Delta\nu_{NO}^{N} = -171$ Hz; $H_{6\beta,7\beta}$:$\Delta\nu_{NO}^{N} = -26$ Hz) structure 2. This assignment is supported by the relative magnitudes of the $\Delta\nu_{NO}^{N}$ values for the β-protons (-101 Hz) and the α-protons (-28.5 Hz) at C(6) and C(7) in isomer 1 and the corresponding values for the axial (-171 Hz) and equatorial protons ($+3$ Hz) at C(2) and C(4) in isomer 2. Different deshielding effects of the N–O bond in the two isomers are also experienced by the bridgehead protons at C(1) and C(5). Since the torsional angle of the N–O and C–H bonds in 1 is smaller than in 2 the bridgehead protons in 1 are more deshielded.

For a quantitative and more detailed analysis of the chemical shifts caused by field effects of the N–O bond in the two isomeric tropine N-oxides and other aliphatic N-oxides calculations using specific parameters for the electric and induced magnetic dipoles of the N–O bond are in progress.

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70. On the Mechanism of Decarboxylation of Betanidine.
A Contribution to the Interpretation of the Biosynthesis of Betalaines

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(3. X. 70)

Zusammenfassung. Bei der Biogenese des Betanins (2) aus Dopa (1) wird die Carboxylgruppe der Aminosäure in die Carboxylgruppe-C(19) umgewandelt. Dieser Schluss beruht auf einem Deuterierungsversuch, bei dem gezeigt wird, dass die Monodecarboxylierung von Betanidin (3) zu einem Verlust von C(19) führt und dass dabei die Doppelbindung C(17)=C(18) nach C(14)=C(15) wandert. Wenn radioaktives Betanidin (3), erhalten aus Einbauexperimenten mit DL-Dopa-[1-^{14}C], in Äthanol decarboxyliert wird, erhält der danach isolierte Dimethylester 7 des Decarboxybetanidin (6)-hydrochlorids nur noch 14% des ^{14}C.

Bei der alkali-katalysierten Äquilibrierung von Betanidin (3) ⇌ Isobetanidin (9) tritt im Gegensatz zur Decarboxylierung keine Wanderung der Doppelbindung ein.

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Previous work has shown that dihydroxyphenylalanine (dopa, **1**) is a precursor in the biosynthesis of betalaines[3] [1]. Experiments with DL-dopa-[1-^{14}C] (**1**) gave betanine (**2**) with 95% of its radioactivity in the three carboxyl groups [2]. Exchange experiments with proline proved that 90% of the radioactivity had been incorporated into the betalamic acid (**4**) portion of the molecule and 10% into the cyclodopa (**5**) part. However, these results did not establish which of the two carboxyl groups

1

2, betanine[3]   **3**, betanidine[3]

4   **5**
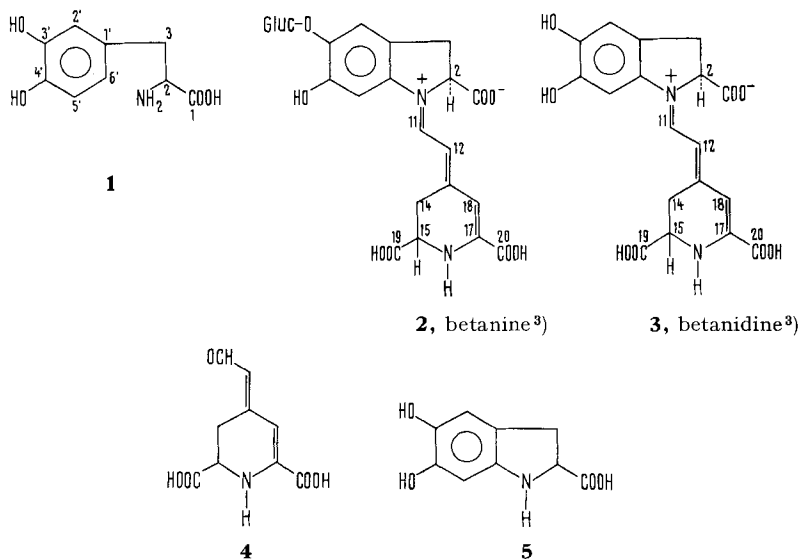
(C(19) and/or C(20)) was the radioactive one. Assuming cleavage of the aromatic ring of dopa and subsequent cyclization[4]) it seemed very likely that the α-amino-acid moiety of dopa (**1**) remained the α-amino-acid moiety (C(19)) of betalamic acid (**4**).

To clarify this point, we turned to the monodecarboxylation of betanidine (**3**) reported by *Minale & Piatelli* [4] in 1965. We confirmed the proposed structure **6** for

A$^-$: anion
(mostly Cl$^-$)

6   **7**

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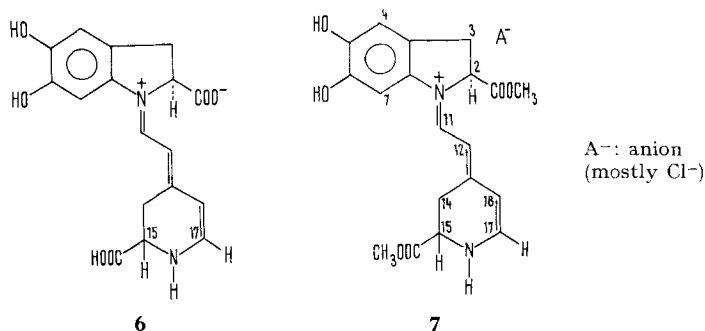
3)   In order to conform with a nomenclature rule of the I.U.P.A.C., from now on we are modifying our previous English orthography of the names betalain, betanin, betanidin, etc. by the addition of a terminal e.

4)   Recent work in our laboratory [3] has shown that the enzymatic cleavage of the aromatic ring of dopa (**1**) occurs between C(4') and C(5').

Reaction Scheme A

3

6
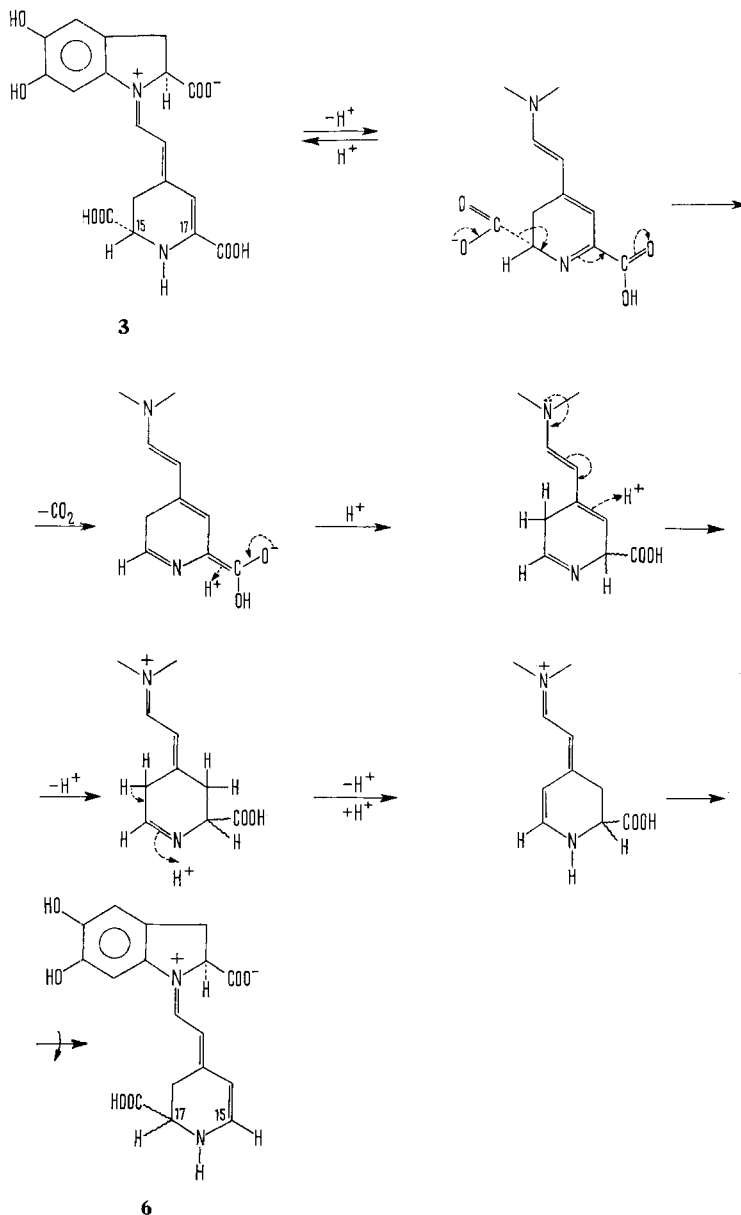
decarboxybetanidine by performing the decarboxylation in boiling ethanol until electrophoretic analysis of microsamples showed the near-absence of betanidine. As decarboxybetanidine (**6**, UV. max. 509 nm) could not be purified readily and did not show a good NMR. spectrum, it was treated with acidic methanol and the resulting dimethyl ester **7** purified by preparative electrophoresis. The UV. (max. 513 nm,

$\varepsilon > 12000$) and the NMR. spectrum (particularly the bands at $\delta = 7.67/d$ ($J = 3$), 1 pr. (H-C(17)), and around $4.7/m$, 1 pr. (H-C(15)) are in accord with structure **7**.

From a simple inspection of the educt (**3**) and product (**6**) formulae it appeared at first sight that this treatment caused the removal of the carboxyl group at C(17) (carbon 20). Therefore the decarboxylation of betanidine-[^{14}C], obtained from the incorporation of DL-dopa-[1-^{14}C] [2], should not result in the loss of any radioactivity. However, when we performed this experiment and esterified the resulting **6**, the purified dimethyl ester **7** contained only 14% of the radioactivity of the betanidine (**3**) [2] used, which, in fact, is only a little more than the radioactivity in the cyclodopa (**5**) portion of the molecule. Thus it appeared that almost *all* the radioactivity of the betalamic acid (**4**) portion had been lost as CO_2.

We were led to consider a mechanism for the monodecarboxylation of betanidine (**3**) which involves loss of the C(19)-carboxyl with concomitant migration of the double bond C(17)=C(18) to C(14)=C(15), and which may correspond to the one shown in reaction scheme A.

To prove this mechanism, betanidine (**3**) hydrochloride was decarboxylated in monodeuteroethanol C_2H_5OD. In the NMR. spectrum of the decarboxybetanidine hydrochloride dimethyl ester thus obtained most of the signal due to the proton at C(15) in **7** ($\delta = 4.7$) was absent, but the signal due to the proton at C(17) ($\delta = 7.67$) remained. The hydrogen at C(15) had been replaced by deuterium (as in **8**) in the enolate quenching step (reaction scheme A). The fact that the proton signal of H-C(17) remained showed that it was not the carboxyl group at the vinyl carbon (C(20) in **3**) which had been lost, for a preliminary protonation (or deuteration) at C(17) in **3** would have been necessary in that case. Thus it is clear that C(15) and C(17) of betanidine (**3**) have become C(17) and C(15) respectively in decarboxybetanidine (**6**) and that the decarboxylation removes C(19) of betanidine.

8
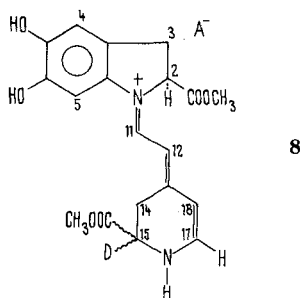
From the evidence for this mechanism and from the above mentioned results of the decarboxylation of radioactive betanidine (**3**) it can be concluded that in the biosynthesis of betanine (**2**), the carboxyl group of dopa (**1**) becomes the α-amino-acid carboxyl group of betalamic acid (C(19) in betanine) and that our proposed biogenetic scheme is correct.

We shall now discuss the mechanism of epimerization of betanidine (**3**). Betanidine isomerizes reversibly with acids and with bases (in absence of oxygen) to isobetanidine (**9**) and the epimerization takes place *exclusively* at C(15) (and not at C(2)) [5]. A mechanism similar to that of the decarboxylation could be considered as responsible for

this reaction (see scheme B), in which the decarboxylation is simply replaced by a presumably much faster deprotonation.

3, betanidine   **9**, isobetanidine
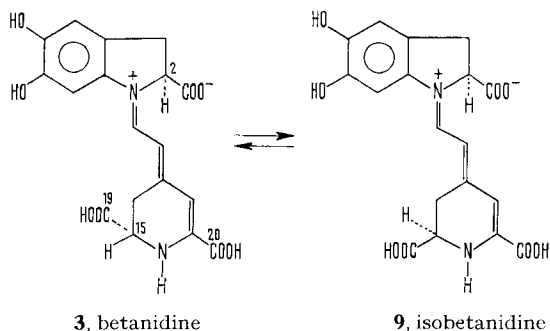
In this case the two carboxyl groups on the dihydropyridine ring (C(19) and C(20)) would become equivalent during the epimerization and our betanidine-[19-^{14}C] (**3**) should be converted into an isobetanidine (**9**) with only about half the radioactivity in C(19).

The above examined radiobetanidine (derived from DL-dopa-[1-^{14}C]) was found to contain 78% betanidine (**3**) and 22% isobetanidine (**9**) (cf. [5]). By treatment with potassium hydroxide in absence of oxygen it yielded a mixture of 40% betanidine (**3**) and 60% isobetanidine (**9**). This mixture was decarboxylated (as hydrochloride) in ethanol. The decarboxybetanidine hydrochloride dimethyl ester (**7**) produced contained about the same radioactivity (approx. 14%) as found in the ester **7** obtained from non base isomerized radiobetanidine (see above). Thus the two carboxyl groups

Reaction Scheme B
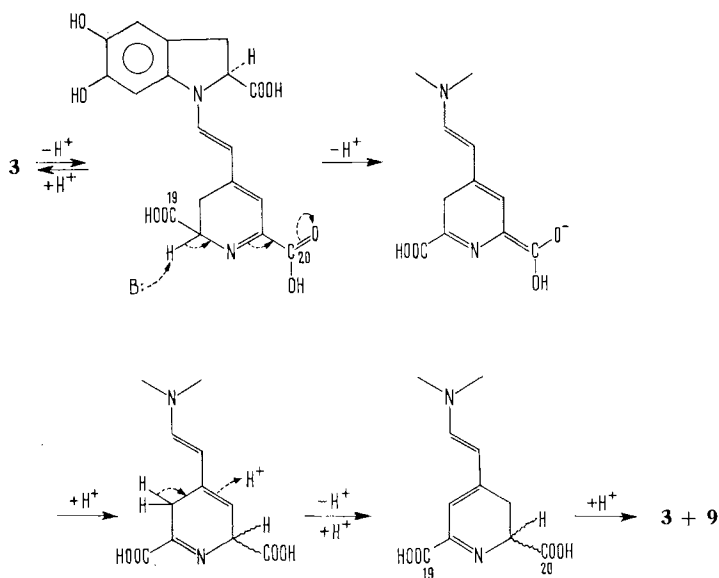
(C(19) and C(20)) had not become equivalent and the above considered mechanism must be excluded.

The fact that epimerization occurs at C(15) rather than at C(2) can nevertheless be explained by the existence of an intermediate of this mechanism; one need simply to assume that the protonation of the intermediate **10** takes place much more rapidly at C(15) than at C(17).
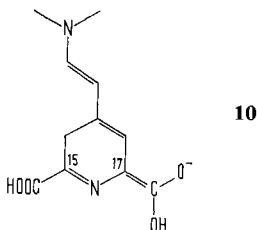
10

Experimental Part

The electrophoretic, chromatographic and spectroscopic methods used in this work as well as the symbols employed for the numerical values are described in [6].

The radioactivity analyses were performed by Mr. *H. Frohofer* and his staff at our microanalytical laboratory [7]. The NMR. spectra (100 MHz) were measured by Mr. *K. Hermann*.

Decarboxybetanidine hydrochloride dimethyl ester (**7**). A solution of 77.6 mg of betanidine (**3**) hydrochloride (90% pure by UV. control [5]) in 100 ml of absolute ethanol was heated under reflux in a stream of purified nitrogen. The progress of the decarboxylation was followed by occasional paper-electrophoretic analysis of the material in a drop of the solution in 0.1 N formic acid. The decarboxy-betanidine (**6**) spot (migration value relative to betanine, $E_b = 0.4$) is well separated from that of betanidine (**3**) ($E_b = 0.7$) and differs in colour (**6**: red; **3**: violet). Boiling was continued until the betanidine spot was weak, which usually took about 90 min. During this time, some decomposition products accumulated which were revealed in the electrophoresis as a dark trail from the starting point. The mixture was filtered and evaporated *in vacuo*. To remove all the ethanol, the residue was dissolved in methanol and again evaporated. The residue of crude decarboxybetanidine (**6**) was esterified with 5 ml of 1 N methanolic HCl at 50° for 15 h in a degassed and evacuated system (cf. [8], p. 1925). The solution of the ester **7** was concentrated and the residue subjected to column electrophoresis on 100 g paper powder (washed with complexone) in 0.1 N formic acid at 1000 V and 30 mA (generally for 21 h). Next to one or two narrow violet bands, the broad red zone of the dimethyl ester **7** was the fastest moving band (several slower moving diffuse red-violet bands were always visible). The paper column was pressed out of the electrophoresis tube and the portion carrying the major red zone was cut out and washed on a *Buchner* funnel with 0.1 N formic acid. The eluate was evaporated in high vacuum to give a thin film of the dimethyl ester **7**, which is assumed to be the chloride; yield 26 mg (38%)[5]. *UV*. (H$_2$O)[6]: Max 512–513 (>12000) nm (ε). *NMR*. (CF$_3$COOH): 8.30/*bm*, 1 pr. (H—C(11)); 7.65/*d* ($J = 3$), 1 pr. (H—C(17)); 7.20/*s*, 1 pr. (H—C(7)); 7.0/*s*, 1 pr. (H—C(4)); 6.2–5.9/*bm*, 2 pr. (H—C(12) & H—C(18)); 5.25/*bm*, 1 pr. (H—C(2)); 4.7/*mb*, 1 pr. (H—C(15)); 4.0/*bs*, 6 pr. (2 × OCH$_3$); 4.0–3.5/*bm*, 4 pr. (2 × H—C(3), 2 × H—C(14)); δ (Hz). In some of the samples additional weak signals were visible. Paper electrophoresis and chromatography [6] (migration values relative to betanine): E_b (pyridinium formate 0.05 N) = -0.9; E_b (formic acid 0.1 N) = -1.8; R_b (pyridinium formate 0.05 N) = 0.6–0.7; R_b (formic

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[5]   This is a maximum yield. The purity of the starting betanidine (**3**) was controlled by UV., but the purity of the product **7** is unknown because a sample of authentic purity is unavailable[6]).

[6]   The decomposition of **7** in aqueous solution is rapid.

acid 0.1 N) = 0.7–0.8. Decarboxybetanidine hydrochloride dimethyl ester (7) appeared as a round spot in the electrophoresis, but as an elongated one in the chromatography. This phenomenon may be due to the presence of two epimers differing in configuration at C(15).

Decarboxybetanidine hydrochloride dimethyl ester (7) *from betanidine-*[^{14}C] (3). A mixture of 8.5 mg radiobetanidine hydrochloride derived from the incorporation of DL-dopa-[1-^{14}C] [2] (95% pure by UV. control [5]) with 3965 dpm/mg and 45.6 mg non-radioactive betanidine hydrochloride (3) (90% pure), thus having average specific activity of 130.9 μCi/mole, was decarboxylated in 75 ml ethanol as described above and yielded 20.3 mg (43%)[5] of dimethyl ester 7 with 90 dpm/mg. The specific activity (16.6 μCi/mole, assuming 7 to be the chloride) corresponds to 12.7% of the specific activity of the starting betanidine. In two other experiments, one with the same sample of radio-betanidine and the other with one of 645 dpm/mg, yields[5] of 35% and 45% of 7 were obtained. The specific activity in both cases was 14% of that of the starting betanidine (3).

Decarboxylation of betanidine (3) *in monodeuteroethanol.* A sample of 43 mg betanidine (3) hydrochloride was decarboxylated in 50 ml C_2H_5OD (Fluka, 99% purity) as described above and yielded 19 mg (51%)[5] of decarboxybetanidine-[D—C(15)] hydrochloride dimethyl ester (8). *NMR.* (CF_3COOH): 8.35/bm, 1 pr. (H—C(11)); 7.65/d (J = 3), 1 pr. (H—C(17)); 7.25/s, 1 pr. (H—C(7)); 7.00/s, 1 pr. (H—C(4)); 6.2–5.9/bm, 2 pr. (H—C(12), H—C(18)); 5.25/bm, 1 pr. (H—C(2)); at 5,0–4,5 (H—C(15)) there was almost no signal; 4.0/bs, 6 pr. (2 × OCH_3); 4.0–3.4/bm, 4 pr. (2 × H—C(3), 2 × H—C(14)); δ (Hz).

Decarboxylation of betanidine (3) *after preliminary basic isomerization.* A mixture of 6.5 mg radiobetanidine hydrochloride derived from the incorporation of DL-dopa-[1-^{14}C] [2] (95% pure) with 3965 dpm/mg and 43 mg non-radioactive betanidine (3) hydrochloride (90% pure), thus having an average specific activity of 110.1 μCi/mole, was isomerized in absence of oxygen with 20 ml of 0.43 N KOH for one hour according to [5]. After acidification with 1 N HCl the mixture was evaporated. The residue was taken up in 0.1 N formic acid and passed through a column (50 × 3.5 cm) filled with 140 g paper powder. Elution with the same solvent gave first a yellow fraction and then a violet one with 24 mg (53%; assay by UV. spectroscopy [5]) of betanidine (partly isomerized). Isomer composition of the radioactive betanidine sample before and after basic isomerization as determined by paper chromatography and UV. measurement (see [5]):

> before basic isomerization   betanidine (3) 78%   isobetanidine (9) 22%
> after basic isomerization   betanidine (3) 40%   isobetanidine (9) 60%

The isomerization was not allowed to reach equilibrium, since this would have resulted in too large a loss [5]. Decarboxylation of the isomerized sample in 30 ml ethanol, as described above, yielded 6 mg (26%)[5] decarboxybetanidine hydrochloride dimethyl ester (7) with 95 dpm/mg. The specific activity 17.3 μCi/mole corresponds to 15.7% of the specific activity of the starting beta-nidine (3).

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