ugation and the supernatant, after being concentrated on the steam-bath, was subjected to paper chromatography for identification of the amino acids released. In most cases two-dimensional chromatography was used with 80% phenol–water as one solvent system and 70% propanol–water as the other. The papers were sprayed with 0.3% ninhydrin in water-saturated *n*-butanol and heated at 105° for 5 min. Controls of enzyme alone and of ovalbumin alone incubated under these experimental conditions were completely negative.

After 30 min. of incubation at 37° the only amino acid that could be demonstrated was alanine. After 60 min. of incubation only alanine could be detected immediately on heating of the paper. However, 24 hr. later very faint traces of several other amino acids became visible. After 120 min. of incubation the yield of alanine as estimated by the spot comparison method[13] was about 20% of the theoretical value calculated on the basis of one mole of alanine per mole of ovalbumin. Because the method of protein coagulation used here probably causes significant losses of free amino acids, this yield can only be taken as a minimum value. At 120 min. five other amino acids could be definitely identified, although present in much smaller amounts than the alanine. According to their R_F values in several solvent systems these were: valine, aspartic acid, glycine, glutamic acid and leucine (or isoleucine).

Subsequent incubations were carried out at 25° in an attempt to slow the reaction and establish, if possible, the sequence of appearance of these extra residues. Again at 25° the only amino acid released in short incubations (1 hr.) was alanine. Longer incubations (5 hr.) yielded larger amounts of alanine together with small amounts of the same five residues mentioned above. In one experiment the only amino acid that could be detected in addition to alanine was aspartic acid.

Since very little detailed information is available concerning the action of carboxypeptidase on intact proteins it was necessary to consider the possibility that the enzyme might be splitting open a cyclic molecule and then removing the C-terminal residue thus made available. If this were the mechanism an N-terminal residue should simultaneously become available (unless, of course, the non-protein moiety formed the bridge between N-terminal and C-terminal residues). The Sanger DNFB method[3] was applied both to native ovalbumin and to carboxypeptidase treated ovalbumin. No N-terminal residue could be demonstrated in either case.

It is concluded that alanine, at least, is a C-terminal residue in ovalbumin. The absence of free α-amino groups,[1,2] which has been confirmed here, is not due to end-to-end cyclization of the molecule but probably due to masking by the non-protein moiety.

It will be noted that the amino acids removed from ovalbumin by carboxypeptidase in the longer incubations are without exception to be found as component amino acids in the small peptides removed from ovalbumin by the *B. subtilis* enzyme of Linderstrøm-Lang and Ottesen.[14,15] While this may be coincidental it suggests very strongly that the peptides cleaved from ovalbumin when it is converted to plakalbumin occupy a C-terminal position in the intact molecule. In support of this hypothesis we have observed that the action of carboxypeptidase on plakalbumin no longer yields alanine predominantly as it does in the case of ovalbumin. Kinetic experiments will be necessary, however, to explore the possibility that one or more of the 5 amino acids appearing subsequent to alanine may also be C-terminal.

(13) R. B. Fisher, D. S. Parsons and G. A. Morrison, *Nature*, **161**, 764 (1948).

(14) K. Linderstrøm-Lang and M. Ottesen, *Compt. rend. trav. Lab. Carlsberg*, **26**, 403 (1949).

(15) M. Ottesen and C. Villee, *ibid.*, **27**, 421 (1951).

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THE VERATRINE ALKALOIDS. XXXIV. THE TRANSFORMATION OF ISORUBIJERVINE TO SOLANIDINE

Sir:

Studies on the tertiary veratrine bases indicate that all those thus far studied are members of a closely related group of hexacyclic substances which includes the potato base solanidine. All possess formulations of 27 carbon atoms and when subjected to selenium dehydrogenation, furnish a characteristic basic degradation product, 2-ethyl-5-methylpyridine.[1-3]

Previous studies on isorubijervine, $C_{27}H_{43}NO_2$ (*Veratrum album*[1,4] and *Veratrum viride*[5]) have shown that this alkaloid possesses a 3(β)-hydroxy-Δ^5-stenol character and the data at hand suggested that it, like rubijervine,[6] may be a hydroxy-solanidine.[7] Recently we have demonstrated this by the direct conversion of isorubijervine to solanidine by a method which appears to avoid any ambiguous stereochemical inversion.

Treatment of isorubijervine [m.p., 236–238°; $[\alpha]^{29}D$ +9.2° (*c* 1.1 in 95% EtOH)], in pyridine with *p*-toluenesulfonyl chloride preferentially yielded a primary mono tosyl derivative (II), m.p. 270–273° dec.; $[\alpha]^{30}D$ −36° (*c* 1.5 in abs. EtOH); *Anal.* Calcd. for $C_{34}H_{49}NO_4S$: C, 71.92; H, 8.70; S, 5.65. Found: C, 71.95; H, 8.58; S, 5.53. II was further characterized by oxidation with aluminum *t*-butoxide to the corresponding Δ^4-3-ketone (III), m.p. 316–319° dec.; *Anal.* Calcd. for $C_{34}H_{47}NO_4S$: C, 72.17; H, 8.37; S, 5.67. Found: C, 71.92; H, 8.37; S, 5.52. Oxime, m.p. 302–304° dec.; *Anal.* Calcd. for $C_{34}H_{48}N_2O_4S$: C, 70.31; H, 8.33; N, 4.82. Found: C, 70.10; H, 8.38; N, 4.82.

Treatment of II with sodium iodide in diethyl

(1) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **148**, 41, 51, 57 (1943).

(2) L. C. Craig and W. A. Jacobs, *Science*, **97**, 122 (1943); V. Prelog and S. Szpilfogel, *Helv. chim. acta*, **25**, 1306 (1942).

(3) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 451 (1943).

(4) *Ibid.*, **159**, 617 (1945).

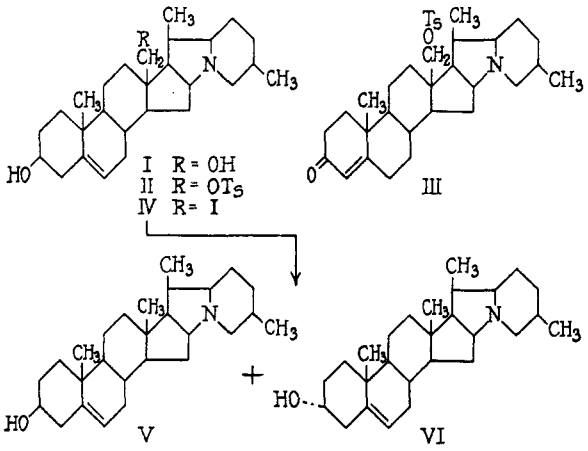
(5) *Ibid.*, **160**, 555 (1945).

(6) *Ibid.*, **179**, 623 (1949).

(7) *Ibid.*, **191**, 63 (1951).

ketone gave an almost quantitative yield of (IV), m.p. 294–297° dec.; $[\alpha]^{30}D$ −38° (*c* 1.0 in abs. EtOH); *Anal.* Calcd. for $C_{27}H_{42}INO$: C, 61.94; H, 8.09; I, 24.24. Found: C, 62.00; H, 8.15; I, 24.12.

Attempts to replace the iodine of IV with hydrogen by treatment with a zinc–copper couple, aluminum amalgam, hydrogen and palladium on calcium carbonate, or with zinc and acetic acid were not successful. However, reduction of IV with sodium in ethanol furnished a good yield of a mixture, m.p. 185–235° dec., which was separated with digitonin into two pure compounds. The first of these (V) was identified as solanidine (3(β)-hydroxy-Δ^5-solanidene), m.p. and mixed m.p. with authentic solanidine, 216–218.5°; $[\alpha]^{34}D$ −27.1° (*c* 0.54 in chf.); *Anal.* Calcd. for $C_{27}H_{43}NO$: C, 81.54; H, 10.91. Found: C, 81.27; H, 10.95. The infrared spectrum of V proved to be identical in all respects with that of authentic solanidine. The second component, m.p. 238–239°, $[\alpha]^{34}D$ −12° (*c* 1.5 in chf.); *Anal.* C, 81.43; H, 11.12, is isomeric with solanidine. Because of its derivation from IV, the similarity of its infrared spectrum to that of solanidine, and its behavior toward digitonin, it is believed to be 3(α)-hydroxy-Δ^5-solanidene (VI). It results presumably from an epimerization accompanying the sodium reduction. Confirmatory work on the structure of VI is in progress.



While the transformation of isorubijervine to solanidine leaves no doubt that isorubijervine is a hydroxy-solanidine, the position of the primary hydroxyl group remains to be settled. The strongly hindered character of the primary iodide group of IV toward the reducing agents cited is in accord with the previous arguments for assigning the hydroxyl group to the 18-position.[7] On the basis of these considerations, irorubijervine is assigned the structure of Δ^5-solanidene-3(β),18-diol (I) and the intermediates the structures represented by II and IV.

All analytical data have been obtained by Mr. D. Rigakos of this laboratory.

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THE NATURE OF THE B–N BOND IN B-TRICHLOROBORAZOLE, BORON NITRIDE AND BORON TRICHLORIDE

Sir:

From their recent structure determination of crystallized $B_3N_3H_3Cl_3$, Coursen and Hoard[1] have shown that the B–N bond length is 1.413 ± 0.01 Å. in this compound. By comparing this value with that found in $B_3N_3H_6$ (1.44 ± 0.02 Å.) they have suggested that double-bond resonance in the B_3N_3 ring must be at least as fully excited in $B_3N_3H_3Cl_3$ as in $B_3N_3H_6$. This implies that the predominant electron configuration must be (A) rather than (B) (Fig. 1), since (B) cannot contribute in $B_3N_3H_6$.

The result obtained for the B–N bond length in boron nitride (1.446 Å.)[2] supports both this contention, and also that of graphite-like resonance in BN, since the observed difference between the two bond lengths (0.033 ± 0.01 Å.) agrees with that expected (0.035 Å.)[3] as a consequence of the required increase in double-bond character of $B_3N_3H_3Cl_3$ as compared to BN.

However, the observed length of the B–Cl bond in $B_3N_3H_3Cl_3$, being the *same* as that observed in BCl_3 to within 0.02 Å.,[1] conflicts with this view: for in BCl_3 the bond is said to be part-double,[4] so that configuration (A) requires a longer B–Cl bond than in BCl_3 by about 0.10 Å. Configuration (B) will certainly not do: it requires not only a *shorter* B–Cl bond than in BCl_3, but also a *longer* B–N bond than in BN.

There is also the possibility of configuration (C). If it is supposed that in BN and BCl_3 there are only single bonds (which are shortened from the sum of Pauling's covalent tetrahedral radii by the deficiency of electrons round the boron), then (C) predicts the same boron radius in all three compounds.

The situation is summarized in Table I.

TABLE I

BOND LENGTHS IN $B_3N_3H_3Cl_3$

Bond	Observed	Predicated by configurations A	B	C
B—N	1.413 ± 0.01	1.411[a]	1.55[a]	1.446[b]
B—Cl	1.760 ± 0.015	1.87[a]	1.65[a]	1.76[b]

[a] Assuming 1/3 part-double-bond in BCl_3 and BN. [b] Assuming single-bond and sextet boron in BCl_3 and BN.

Clearly, neither (A) nor (B) can alone account for the bond lengths observed in $B_3N_3H_3Cl_3$. However, a combination of two-thirds (A) and one-third (B), which gives a one-third part-double character to both the B–Cl and the B–N bond, will, on the assumption of similar bonds in BN and BCl_3, give as good a fit as (C).

There is likely, however, to be some real difference between the B–N bond in BN and in $B_3N_3H_3Cl_3$ to account for the observed difference in length. This may be a difference between the contribution from (C) in the two substances. Without definite information on the bond type in BN and BCl_3, the bond lengths do not yield infor-

(1) D. L. Coursen and J. L. Hoard, THIS JOURNAL, **74**, 1742 (1952).
(2) R. S. Pease, *Acta Cryst.*, **5**, 356 (1952).
(3) L. Pauling, *Proc. Roy. Soc.* (*London*), *A* **196**, 343 (1949).
(4) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1945.