around 10% less than that reported for the monomer of the common strain.[3]

The Kerr constant increased a factor of about 20 as the *p*H was increased from 5.6 to 8.3. The variation resembled a titration curve with an inflection around *p*H 7. Experiments with the square reversing pulse as a function of *p*H indicated that the permanent moment contribution also increased with *p*H. The amino acid analyses, which have been reported[4] for all four strains mentioned above, show that histidine is present only in the Rib Grass strain. Titration of the imidazole group of histidine occurs around *p*H 7, and there is no other known constituent which is titrated in this region.[4,5] This leads to the suggestion that an asymmetric arrangement of histidine residues in the macromolecular structure might be the origin of the large changes of dipole moment and Kerr constant around *p*H 7.

The lower rotation diffusion constant cannot be explained by rigid end-to-end dimers, which were suggested for the common strain.[3] It is the value expected for a staggered side-by-side dimer in which the monomer units overlap about one-half of their lengths, but it may be due to other types of aggregates. Analysis of the transient behavior of the birefringence, which is complicated by the presence of two components, leads to the tentative conclusions that both components have permanent dipole moments, and that they are *ca.* 10^4 to 10^5 debye units.

A more complete account of this work is available elsewhere.[6] Further studies are in progress on the macromolecular properties as a function of preparation procedure.

We are happy to acknowledge financial support from the National Science Foundation and the California Research Corporation.

(3) C. T. O'Konski and A. J. Haltner, THIS JOURNAL, **78**, 3604 (1956).

(4) C. A. Knight, *J. Biol. Chem.*, **171**, 297 (1947).

(5) R. A. Alberty in H. Neurath and K. Bailey, "The Proteins," Vol. 1, Part A, Chapter 6, Academic Press, Inc., New York, N. Y., 1953.

(6) Details are presented in the Ph.D. Thesis of R. M. Pytkowicz, June, 1957, University of California, Berkeley, California.

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RECEIVED JUNE 21, 1957

THE *p*H OPTIMUM OF THE HILL REACTION

Sir:

One of the contradictions in the Hill reaction literature is that concerning the *p*H optimum at high light intensity. The "optimal" values previously cited range from *p*H 5.9 to 8.3 (Table I). As will be shown in this paper, this discrepancy is largely due to the fact that the *p*H optimum is dependent on the activity of the chloroplast material. A high *p*H optimum is characteristic of high activity preparations, and the optimum decreases as the chloroplasts lose activity.

In the experiment from which the following data were taken, washed, turgid leaves of field grown chard (*Beta vulgaris*) were used as the source of chloroplasts. The leaves were ground with a mortar and pestle in the presence of 0.03 *M* phosphate buffer, *p*H 7.0, containing 0.01 *M* KCl. The suspension was filtered through glass wool, then centrifuged for 90 seconds at 100 *g* to remove whole cells and other debris. It was then decanted and recentrifuged for five minutes at 900 *g* to bring down "whole chloroplasts." The supernatant was discarded, the centrifuge tube and the surface of the chloroplast pellet were rinsed with fresh, cold buffer, and the inside of the tube was wiped dry. The chloroplasts were resuspended by gentle stirring during slow addition of fresh buffer of the same composition as the grinding medium. All operations were carried out as close to 0° as possible, and no effort was made to avoid exposing the preparation to either light or air.

TABLE I

*p*H OPTIMA FOR THE HILL REACTION AS REPORTED BY DIFFERENT WORKERS

Holt, Smith and French[1]	5.9
Warburg and Lüttgens[2]	6.5
Wessels[3]	6.5
Arnon and Whatley[4]	6.9
Spikes, Lumry, Eyring and Wayrynen[5]	6.9
Punnett and Fabiyi[6]	6.9
Clendenning and Gorham[7]	7.15
Holt and French[8]	7.6–7.9
Punnett[9]	7.9
Hill and Scarisbrick[10]	8.0
Davenport, Hill and Whatley[11]	8.3

Reaction rates were measured by following the rate of reduction of purified 2,6-dichlorophenolindophenol (molar extinction coefficient 22,400).[9,12] The reaction vessel contained 0.03 *M* phosphate or Tris buffer, 0.01 *M* KCl, 1.5×10^{-5} *M* dye, and chloroplasts giving a final concentration of chlorophylls *a* plus *b* of 3.5 μM./l (3.2 μg./ml.). In the dark the rate of reduction of the dye by the chloroplasts corresponded to a $Q_{O_2}^{ch}$ of 30, which is roughly the same as the error in the determination of the rate in high intensity red light.

When freshly prepared chloroplasts were tested for *p*H response within one to two hours after grinding the leaves, the optimum was found to be equal to or greater than *p*H 8.7. Following storage of the preparation at 0°, the *p*H optimum decreased half a *p*H unit during the first nine hours of storage, and another unit and a half during the next 43 hours (Table II).

(1) A. S. Holt, R. F. Smith and C. S. French, *Plant Physiol.*, **26**, 164 (1951).

(2) O. Warburg and W. Lüttgens, *Biokhim.*, **11**, 303 (1946).

(3) J. S. C. Wessels, "Investigations into Some Aspects of the Hill Reaction," Thesis, University of Leyden, 1954.

(4) D. I. Arnon and F. R. Whatley, *Arch. Biochem.*, **23**, 141 (1949).

(5) J. D. Spikes, R. W. Lumry, H. Eyring and R. Wayrynen, *ibid.*, **28**, 48 (1950).

(6) T. Punnett and A. Fabiyi, *Nature*, **172**, 947 (1953).

(7) K. A. Clendenning and P. R. Gorham, *Can. J. Res.*, **C28**, 78 (1950).

(8) A. S. Holt and C. S. French, *Arch. Biochem.*, **9**, 25 (1946).

(9) T. Punnett, Revues des Fermentations, 1956; paper presented at 3rd Int. Cong. Biochem., Brussels, 1955.

(10) R. Hill and R. Scarisbrick, *Proc. Roy. Soc.*, **B129**, 238 (1940).

(11) H. E. Davenport, R. Hill and F. R. Whatley, *ibid.*, **B139**, 346 (1952).

(12) N. Savage and T. Punnett, unpublished results.

TABLE II

SHIFT IN *p*H OPTIMUM WITH DECREASING ACTIVITY

Time after grinding leaves, hours	*p*H optimum	$Q_{O_2}^{ch}$
1–2	≥8.70	2150
5–6	8.40	1670
10–11	8.25	1370
34–35	flat response	590
53–54	6.85	250

It should be pointed out that the extremely high initial *p*H optimum (≥8.7) was associated with exceptional stability of the chloroplasts, for it was found with only three different chard chloroplast preparations made during one week of July, 1956. Usually the *p*H optimum of highly active chloroplasts ($Q_{O_2}^{ch}$ 1800 or higher) is between 8.0 and 8.5, and decreases with decreasing activity. With a few exceptions the optimum does not depend on the species of plant used as the source of chloroplasts. The foregoing illustrates the importance of expressing the activity in absolute terms ($Q_{O_2}^{ch}$) when determining the *p*H optimum for any preparation.

When these results are compared with those obtained by others, it is found that in all cases low *p*H optima are associated with low activity,[2,5,6] or with prolonged preincubation of the chloroplasts in alkaline medium[1,3,4,7] which is known to inactivate them,[9] or with both.

In contrast to the results obtained when using high intensity light, the *p*H response for the Hill reaction in light of low intensity (light limited reaction) proves to be flat from *p*H 6.35 to 8.40.

A more complete description of the results reported here and earlier[9] will be published elsewhere.

This work was done while the author was a postdoctoral fellow of the National Foundation for Infantile Paralysis at the School of Biochemistry, Cambridge, England.

The author wishes to express his appreciation to Dr. R. Hill for his help and advice during the course of this investigation.

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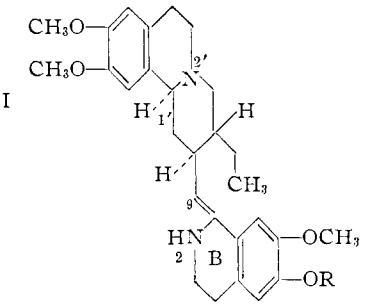
RECEIVED JUNE 5, 1957

STEREOCHEMISTRY OF THE IPECAC ALKALOIDS

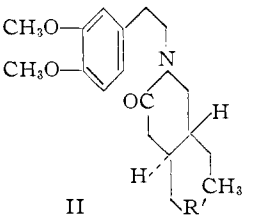
Sir:

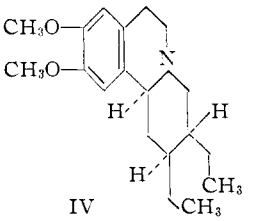
Despite the intensive and thorough investigations carried out on the alkaloids of Ipecac root,[1] including the reported total synthesis[2] of the best-known member of the family, *l*-emetine, there has not been available any direct evidence bearing on the stereochemical aspects of these important bases. Largely through the correlation of an intermediate used in the total synthesis[2] with a reference compound of proved stereochemistry, it is now possible to propose the stereoformula I[3] for psychotrine (R = H), O-methylpsychotrine (R = CH_3), cephaëline (R = H, 1,9-double bond saturated), emetamine (R = CH_3, ring B aromatic) and emetine (R = CH_3, 1,9-double bond saturated), all members of the same stereochemical family.[1]

I



Alkylation of 3,4-dimethoxy-β-phenethylamine with ethyl *dl-threo*-3,4-diethyl-5-bromovalerate[4] yields the reference compound, the lactam (II, R′ = CH_3) of *dl*-N-(3,4-dimethoxy-β-phenethyl)-*threo*-3,4-diethyl-5-aminovaleric acid, b.p. 128–130° (0.1 mm) (Found: C, 71.15; H, 8.92). Phosphorus oxychloride cyclization to the unisolated 3,4-dihydroisoquinolinium cation (III), followed by saturation of the carbon–nitrogen double bond (*vide infra*) provided the tetrahydroisoquinoline (IV) (hydrochloride, m.p. 247.5–248.5°. Found: C, 66.70; H, 8.57). This same[5] pair of substances was obtained from the emetine synthesis intermediate II (R′ = $COOC_2H_5$)[2] by (i) controlled reduction with lithium borohydride, which afforded the lactam alcohol II (R′ = CH_2OH), m.p. 116–117° (Found: C, 68.20; H, 8.65), followed by (ii) conversion to the tosylate and thence to the isothiouronium salt, which, without purification, was reductively desulfurized by treatment with Raney yielding *dl*-II (R′ = CH_3); transformation to the *dl*-base IV confirmed the identity of this intermediate.





Catalytic hydrogenation of the 3,4-dihydroisoquinolinium salt III to the tetrahydro-base IV involves axial attachment of hydrogen, since sodium-ethanol reduction also yields IV. Therefore, emetine also appears to possess the more stable configuration at C-1′, as depicted in formula I, since the synthetic *dl*-alkaloid is obtained[2] by catalytic reduction of the di-salt (V) of *dl*-I (R = CH_3) $\Delta^{1'(2')}$ and $\Delta^{1(2)}$ rather than $\Delta^{1(9)}$ [6]; this view is supported by the observation that the same reduction

(1) M.-M. Janot, R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. III, Academic Press, Inc., New York, 1953, pp. 363–394.

(2) R. P. Evstigneeva, R. S. Livshits, M. S. Bainova, L. I. Zakharkin and N. A. Preobrazhenskii, *J. Gen. Chem.*, **22**, 1467 (1952).

(3) Formula I bears no implication as to absolute configuration.

(4) E. E. van Tamelen, P. E. Aldrich and T. J. Katz, *Chem. and Ind.*, 793 (1956).

(5) On the basis of appropriate infrared spectral comparisons and a m.m.p. on the hydrochloride salts of IV.

(6) Our repetition of the Russian work (ref. 2) indicates that isoemetine (epimeric with emetine at C-1) is also produced in this step.