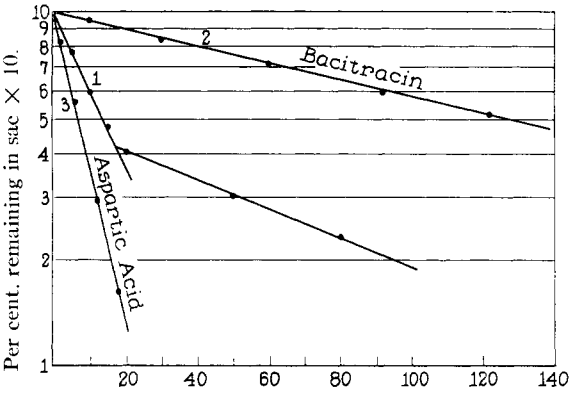


Fig. 2.

different membranes prepared from the same roll appear remarkably reproducible. The most permeable one thus far studied (No. 20/32 "Dialysis tubing")[3] has been found to pass insulin, ribonuclease, lysozyme and chymotrypsinogen in 0.1 *N* acetic acid at characteristically different rates (50% escape times in same cell are 1.6, 3.5, 3.5 and 5 hr., respectively). The membrane permeability can be increased by mechanical stretching so that ovalbumin readily passes.

A full account of this work will be reported soon.

(3) Light and Simpson, *Biochem. Biophys. Acta*, **20**, 251 (1956), found the 20/32 size to pass insulin.

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.
LYMAN C. CRAIG
TE PIAO KING

RECEIVED JULY 5, 1956

THE STRUCTURE OF OXIMES

Sir:

Pitt,[1] in a review article, has suggested that oximes should be represented as $R_2C{=}{}^{+}NH{-}O^{-}$ rather than, as ordinarily written, $R_2C{=}N{-}OH$. However, the weight of more recent evidence, set forth below, favors the classical formulation of $R_2C{=}N{-}OH$. The basis for Pitt's suggestion was a preliminary report of the crystal structure determination of *syn-p*-chlorobenzaldoxime.[2] The hydrogen bonding in this crystal was said to be: N. . .O distances of 2.82 Å., with the angles: O–N . . .O′ = 101.4° and N–O. . .N′ = 82°. Since the former is closer to that expected for a covalent bond angle (the hydrogen atom is assumed to lie on or near the N. . .O′ and O. . .N′ axes), the structure $R_2C{=}{}^{+}NH{-}O^{-}$ is indicated. The final results of this structure determination have not yet been published. Dunitz and Robertson[3] later discussed Pitt's suggestion, pointing out that the structure found for acetoxime,[4] with angles O–N. . .O′ of 124° and N–O. . .N′ of 111°, was compatible with either structure, but the results in the case of dimethylglyoxime[5] probably supported Pitt's suggestion. As pointed out by Dunitz and Robertson,[3] Merritt and Lanterman's dimethylglyoxime paper contains an obvious error, since the latter authors stated that both of the above angles equal 75.9°, an impossible situation since the two oxime groups are related by a center of symmetry; Dunitz and Robertson then assumed, apparently by inspection of the published projection of the structure on (001), that the angle N–O. . .N′ was smaller and equal to 75.9°, and that the angle O–N. . .O′ was its supplement, and therefore closer to that expected for the covalent bond. Unfortunately, there is yet another error, for the published parameters give instead for these angles 85° for N–O. . .N′ and 95° for O–N. . .O′, and these are close enough together to cause the argument to lose considerable force.

Additional information relative to this question has appeared recently, namely, the results of the crystal structure of formamidoxime.[6] For this molecule the situation is somewhat more complicated because there are more than just the two tautomeric structures possible. Nevertheless, detailed considerations show that the hydrogen bonding in this crystal is consistent with only two structures, I: $NH_2{-}CH{=}N{-}OH$, and II: $^{-}NH{-}CH{=}N{-}{}^{+}OH_2$. (Each of these has more than one resonance form.) The structure $NH_2{-}CH{=}{}^{+}NH{-}O^{-}$ is in particular eliminated because both atoms which form hydrogen bonds with the oxime nitrogen atom lie so far from the molecular plane that this atom must be the acceptor atom in these two hydrogen bonds. Structure II above, may be rejected[6] on the ground that the relative electronegativities of oxygen and nitrogen will render both of its resonance forms unstable. We are thus left with Structure I, and the observed bond lengths indicate that the formamidoxime molecule is best represented as a resonance hybrid, the predominant forms being $NH_2{-}CH{=}N{-}OH$ and $^{+}NH_2{=}CH{-}N^{-}{-}OH$, which contribute about equally, and perhaps a small contribution of the form $NH_2{-}{}^{-}CH{-}N{=}{}^{+}OH$.

At present, therefore, it appears that the usual oxime structure, $R_2C{=}N{-}OH$, is correct, but obviously a direct location of the hydrogen atoms in an oxime by use of accurate three dimensional X-ray data, or by neutron diffraction, would be highly desirable.

(1) G. J. Pitt, *Annual Reports of the Chemical Society*, **47**, 457 (1950).
(2) B. Jerslev, *Nature*, **166**, 741 (1950).
(3) J. D. Dunitz and J. H. Robertson, *Annual Reports of the Chemical Society*, **49**, 378 (1952).
(4) T. K. Bierlien and E. C. Lingafelter, *Acta Cryst.*, **4**, 450 (1951).
(5) L. L. Merritt and E. Lanterman, *ibid.*, **5**, 811 (1952).
(6) D. Hall and F. J. Llewellyn, *ibid.*, **9**, 108 (1956).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA
JERRY DONOHUE

RECEIVED APRIL 9, 1956

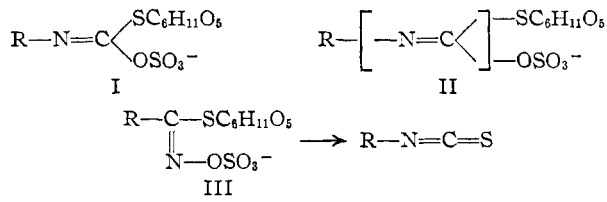
THE STRUCTURES OF SINIGRIN AND SINALBIN; AN ENZYMATIC REARRANGEMENT

Sir:

The myronate ion, isolated as the potassium salt sinigrin in 1839, is the precursor of the isothiocyanate of black mustard and horseradish and the prototype of mustard oil glucosides. The currently accepted structure (I, R = $H_2C{=}CHCH_2$), proposed in 1897,[1] rested on the enzymatic hydrolysis of sinigrin to allyl isothiocyanate, D-glucose and bisulfate ion, the cleavage by silver nitrate to glucose and silver sinigrate, $C_4H_5O_4NS_2Ag_2$, a mer-

(1) J. Gadamer, *Arch. Pharm.*, **235**, 44 (1897).

captide containing the elements of sulfate and allyl isothiocyanate, and subsequently on the alkaline hydrolysis of sinigrin to β-D-1-thioglucopyranose.[2] However, I does not accord with the known facts[1,3,4] that sinigrin and analogs yield on direct chemical degradation, not the amines (RNH_2) expected from I, but nitriles (RCN) and carboxylic acids containing the same number of carbon atoms as the enzymatically formed isothiocyanates (RNCS).



I II III

The preceding evidence did establish the general formula II. We now show that version III is the proper expression for the mustard oil glucosides[5]: namely, the decompositions of III or the corresponding silver mercaptide to the nitrile parallel known[6] fissions of thiohydroxamic acids; the formation of the isothiocyanate concurrently with enzymatic removal of the glucosyl group, or nucleophilic abstraction of silver from the mercaptide,[1,3] though novel as an enzyme-initiated process, is an analog of the Lossen rearrangement.[7]

We have proved structure III for sinigrin (R = $H_2C{=}CHCH_2$) and for the glucosinalbate ion (R = p-$HOC_6H_4CH_2$), which is obtained as sinalbin (sinapine glucosinalbate, isolated in 1831) from yellow mustard and furnishes p-hydroxybenzyl isothiocyanate on enzymatic hydrolysis. The glucosinalbate ion was studied as the anhydrous tetramethylammonium salt, m.p. 191–192° (dec.), $[\alpha]^{35}D$ −19° (in water). With Raney nickel in water at room temperature, sinigrin furnished n-butylamine, isolated in 47% yield as the p-nitrobenzamide, and the glucosinalbate furnished tyramine, isolated in 37% yield as the hydrochloride. On acid hydrolysis, sinigrin gave vinylacetic acid, and the glucosinalbate, p-hydroxyphenylacetic acid. Conclusively, acid hydrolyses of sinigrin, sinalbin or the glucosinalbate also afforded *hydroxylamine* in 50–90% yields.[8] Hydrochloric acid at room temperature was effective, but we have mostly used a modification of Yamada's assay[9] for bound hydroxylamine, cleavage in 3 M sulfuric acid containing 2,4-dinitrophenylhydrazine for 2 hours at 95–100°. The hydroxylamine liberated was identified by paper chromatography[10] ($R^{30°F.}$ 0.46–0.51 in 7:3 methanol–6 N hydrochloric acid, detected by three specific reagents), determined quantitatively by the Csaky–Yamada method[9] or as ferric benzhydroxamate,[11] and isolated as fluorenone oxime, m.p. 189–191°, in 13–47% yields from sinigrin and the glucosinalbate.[12]

We conclude: that hydroxylamine derivatives can be accumulated in higher plants[13]; that the skeletal resemblance of amino acids and mustard oil glucosides (*cf.* tyrosine and glucosinalbate) is clarified[14]; that mustard oils are formed in nature by an enzyme-actuated Lossen rearrangement.[14]

We thank Mr. R. W. King, R. T. French Company, for gifts of mustard flour, the National Science Foundation for a predoctoral fellowship (A. J. L.), and The Robert A. Welch Foundation for financial support.

(2) W. Schneider, H. Fischer and W. Specht, *Ber.*, **63**, 2787 (1930). The complete formulation of sinigrin as a β-glucopyranoside is confirmed by the rotation and our desulfurization of tetraacetylsinigrin to tetraacetyl-1,5-anhydro-D-glucitol.

(3) H. Will and W. Koerner, *Ann.*, **125**, 257 (1863); J. Gadamer, *Arch. Pharm.*, **237**, 111, 507 (1899); H. Schmid and P. Karrer, *Helv. Chim. Acta*, **31**, 1017, 1087 (1948).

(4) H. Will and A. Laubenheimer, *Ann.*, **199**, 150 (1879); O. E. Schultz and R. Gmelin, *Arch. Pharm.*, **287**, 342 (1954).

(5) We reject the third possibility, attachment of the thioglucosyl group to the nitrogen atom, because a substance so constituted could afford the isothiocyanate only by an obscure *twofold* shift and would give ammonia on acid hydrolysis, cleaving like known sulfenamides (N. Kharasch, S. J. Potempa and H. L. Wehrmeister, *Chem. Revs.*, **39**, 269 (1946)).

(6) L. Cambi, *Atti reale accad. Lincei, Rend. classe sci. fis., mat. e nat.*, [5] **18**, I, 687 (1909); L. Voltmer, *Ber.*, **24**, 378 (1891).

(7) *Cf.* R. D. Bright and C. R. Hauser, THIS JOURNAL, **61**, 618 (1939). The sulfate residue in III is assigned the configuration *anti* to R, the migrating group.

(8) *Cf.* hydrolyses of ketoxime-O-sulfonic acids: F. Sommer, O. F. Schulz and M. Nassau, *Z. anorg. u. allgem. Chem.*, **147**, 142 (1925); P. A. S. Smith, THIS JOURNAL, **70**, 323 (1948); D. E. Pearson and F. Ball, *J. Org. Chem.*, **14**, 118 (1949).

(9) T. Yamada, *Acta Chem. Scand.*, **9**, 349 (1955).

(10) J. M. Bremner, *Analyst*, **79**, 198 (1954).

(11) G. W. Pucher and H. A. Day, THIS JOURNAL, **48**, 672 (1926).

(12) Progoitrin (M. A. Greer, *ibid.*, **78**, 1260 (1956)), kindly supplied by Dr. Greer, afforded hydroxylamine in 67% assayed yield.

(13) The sinigrin in black mustard is equivalent to a hydroxylamine content of the seed up to *ca.* 0.4%.

(14) Isoleucine and natural (+)2-butyl isothiocyanate, which apparently belong to the same stereochemical series, and the corresponding glucoside could presumably be correlated through (+)2-methylbutyric acid. In the enzymatic formation of this isothiocyanate the migrating carbon atom is asymmetric, and should retain configuration!

DEPARTMENT OF CHEMISTRY
W. M. RICE INSTITUTE MARTIN G. ETTLINGER
HOUSTON 1, TEXAS ALLAN J. LUNDEEN

RECEIVED JULY 5, 1956

THE SYNTHESIS OF ANALOGS OF THE AMINONUCLEOSIDE FROM PUROMYCIN: VARIANTS AT THE 6-POSITION OF THE PURINE MOIETY

Sir:

Since 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine (I, R_1 = $N[CH_3]_2$),[1] the aminonucleoside from puromycin[2,3,4] has trypanocidal[5] and tumor-inhibiting properties,[6] analogs were synthesized in which substituents in the 6-position of the purine moiety were varied in order to determine the relation of structure to activity. The synthesis of the 6-amino analog, 3′-amino-3′-deoxyadenosine, from 6-benzamidopurine has been described by Baker, *et al.*[7]

(1) B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **76**, 2838 (1954).

(2) Stylomycin is the registered trade-mark of the American Cyanamid Company for the antibiotic puromycin.

(3) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos and J. H. Williams, *Antibiotics and Chemotherapy*, **2**, 409 (1952).

(4) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

(5) R. I. Hewitt, A. R. Gumble, W. S. Wallace and J. H. Williams, *Antibiotics and Chemotherapy*, **4**, 1222 (1954).

(6) P. L. Bennett, S. L. Halliday, J. J. Oleson and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 766–769.

(7) B. R. Baker, R. E. Schaub and H. M. Kissman, THIS JOURNAL, **77**, 5911 (1955).