enzymes.[25-27] Finally, the striking catalytic effect of small concentrations of barium ion on the hydrolysis of copolymer A/An and its interpretation in terms of the elimination of an inhibitory neighboring group effect adds yet another possible mechanism to those enumerated previously[28] for the activation of enzymes by specifically required ions.

In many cases low-molecular weight model compounds should serve as well or better than polymers in such model studies. However, copolymerization is a very simple technique compared to the rather complex synthetic problems that might otherwise have to be faced. In addition, the use of polymers with a large number of hydrophilic groups makes it possible to keep in aqueous solution groupings which would otherwise be too insoluble for study. Both points may be strikingly illustrated on the barium catalysis of A/An hydrolysis. To demonstrate a similar effect on a low molecular weight compound, it would be necessary to study the hydrolytic rates of the structures III and IV.

It is clear that these materials would be very hard to prepare and that the chelate IV would be too insoluble for a kinetic study.

BROOKLYN 1, N. Y.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

# The Reaction of D-Glycerose-3-$C^{14}$ with Alkali[1]

BY JOHN C. SOWDEN AND EVA K. POHLEN

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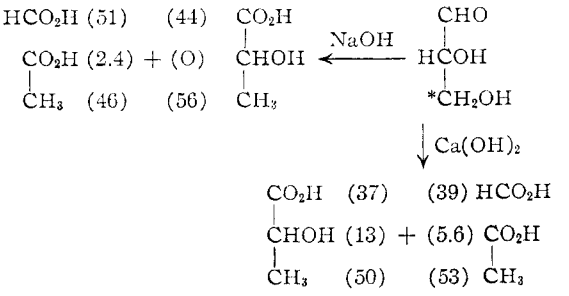
The isomerization of D-glycerose-3-$C^{14}$ by 1.68 *N* sodium hydroxide at 25° leads to lactic acid labeled almost equally, and only, in C-1 and C-3. In contrast, saturated calcium hydroxide at 25° converts D-glycerose-3-$C^{14}$ to lactic acid with significant amounts of label in all three carbon atoms. An interpretation of these results is offered in terms of the Nef–Isbell mechanism for the formation of saccharinic acids.

In their classical studies[2] of the interconversion of reducing sugars by alkali, Lobry de Bruyn and Alberda van Ekenstein recognized certain variations in the details of the reaction in the presence of different bases. Such differences have been attributed[3] more recently to specific, cationic effects of the reacting bases.

Different bases also have been observed to produce different products, although under very different reaction conditions, in the isomerization of reducing sugars to saccharinic acids. Thus, D-glucose with calcium hydroxide at room temperature gives "α"-D-glucosaccharinic acid as the principal six-carbon saccharinic acid,[4] whereas the same sugar with hot 8 *N* sodium hydroxide is reported to give no trace of this acid, but rather the D-glucometasaccharinic acids plus a small amount of "α"-D-isosaccharinic acid.[5] These results have been attributed[6] recently to variations in the ionization behavior of the intermediate sugar enediols at different *p*H values, and a resultant channeling of the reaction into one or other of the alternative pathways of the Nef–Isbell mechanism.[5,7]

In the present continuation of our studies[8] on saccharinic acid formation from $C^{14}$-labeled sugars, the action of saturated calcium hydroxide and of 1.68 *N* sodium hydroxide at 25° on D-glycerose-3-$C^{14}$ have been examined in an attempt to elucidate further the course of the reaction in the presence of different bases. The lactic, acetic and formic acids[9] obtained in each case were separated by chromatography and radioassayed in the form of appropriate derivatives. The acetic acid was degraded[8,10] by way of 2-methylbenzimidazole, 2-styrylbenzimidazole and benzimidazole-2-carboxylic acid to benzimidazole, and the lactic acid[10] by way of 2-(α-hydroxyethyl)-benzimidazole and benzimidazole-2-carboxylic acid to benzimidazole, to obtain values for the relative distribution of radioactivity among the various carbon atoms. The percentages of the specific radioactivity of the lactic acid found in the individual carbon atoms of the products is shown in parentheses in the accompanying formulas.




With 1.68 *N* sodium hydroxide, D-glycerose-3-$C^{14}$ yielded lactic acid labeled nearly equally, and en-

(1) Abstracted from a thesis submitted by Eva K. Pohlen in partial fulfillment of the requirements for the degree Master of Arts, Washington University, June, 1954.
(2) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895); **15**, 92 (1896); **16**, 257, 262, 282 (1897); **18**, 147 (1899); **19**, 1 (1900).
(3) A. Kuzin, *Ber.*, **68**, 619, 1494 (1935); **69**, 1041 (1936), J. C. Sowden and R. Schaffer, THIS JOURNAL, **74**, 499 (1952).
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(8) J. C. Sowden, M. G. Blair and D. J. Kuenne, THIS JOURNAL, **79**, 6450 (1957).
(9) W. L. Evans and H. B. Hass, *ibid.*, **48**, 2703 (1926).
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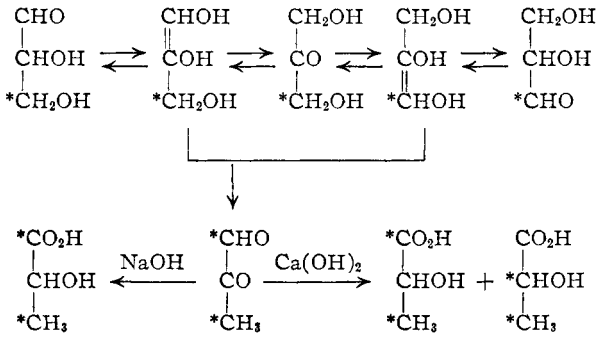
tirely, in C-1 and C-3. A similar distribution of label had been observed previously by Gibbs[11] in the lactic acid formed from the reaction of D-glucose-1-C$^{14}$ with 3 *N* potassium hydroxide at 50°. In contrast, the lactic acid formed from D-glycerose-3-C$^{14}$ and calcium hydroxide contained significant amounts of label in all three carbon atoms. A similar randomization of the label also has been observed[8] in the lactic acid resulting from the action of calcium hydroxide at room temperature on D-mannose-1-C$^{14}$. The present results with D-glycerose-3-C$^{14}$ can be interpreted in terms of the Nef–Isbell mechanism for saccharinic acid formation as follows. It may be assumed that the reversible isomerization of glycerose with dihydroxyacetone, in the presence of either base, is fast in comparison to the rate of saccharinic acid formation. Hence, the lactic acid is formed essentially from triose




enediol labeled equally at C-1 and C-3. The α-dicarbonyl intermediate of the Nef–Isbell mechanism is, in this instance, pyruvaldehyde,[12] which is subject to migration either of the methyl group (saccharinic mechanism) or of a hydrogen atom (metasaccharinic mechanism). The latter migration appears to be exclusive in the presence of sodium hydroxide to produce lactic acid labeled in C-1 and C-3. The migration of the hydrogen atom also preponderates with calcium hydroxide, but some migration of the methyl group also occurs to form lactic acid labeled in C-2 and C-3. A combination of about 75% hydrogen atom migration and 25% methyl group migration in the calcium hydroxide experiment would produce lactic acid with the labeling pattern found by radioassay.

A consideration of the labeling patterns found in the acidic products suggests common carbon sources within the glycerose chain for the following pairs: (a) formic acid and C-1 of lactic acid, (b) C-1 of acetic acid and C-2 of lactic acid and (c) C-2 of acetic acid and C-3 of lactic acid. Thus, the cleavage of the three-carbon chain that gives rise to the acetic and formic acids, and which cannot occur by the Schmidt[13] mechanism, appears to be an alternative reaction pathway in the rearrangement of pyruvaldehyde to lactic acid.[9]

The condensation of triose to hexose in alkaline solution has long been known.[14] Accordingly, six-carbon saccharinic acids are to be expected among the products of the prolonged action of alkali on glycerose. In the present work, "α"-D-glucosaccharinic lactone was isolated in low yield from the D-glycerose–calcium hydroxide reaction mixture.

(11) M. Gibbs, THIS JOURNAL, **72**, 3964 (1950).

(12) Pyruvaldehyde has been identified in alkaline solutions of glycerose by V. Prey and E. Waldman, *Monatsh.*, **83**, 65 (1952).

(13) O. Schmidt, *Chem. Revs.*, **17**, 137 (1935).

(14) E. Fischer and J. Tafel, *Ber.*, **20**, 1088, 2566 (1887); E. Schmitz, *ibid.*, **46**, 2327 (1913); H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **19**, 519 (1936).

## Experimental

**D-Glycerose-3-C$^{14}$.**—Eighty grams of D-mannitol, containing 100 μc. of D-mannitol-1,6-C$^{14}$,[15] was converted to D-glycerose-3-C$^{14}$ ([α]$^{22}$D 14° in water) by the method of Baer and Fischer.[16] The intermediate 1,2:5,6-di-*O*-isopropylidene-D-mannitol-1,6-C$^{14}$ (74 g., m.p. 119°) showed specific radioactivity[17] of 3.6 × 10$^5$ cts./min./mM.

**Isomerization of D-Glycerose-3-C$^{14}$.**—A solution (50 ml.), 1.68 *N* in sodium hydroxide and containing 6.17 g. of D-glycerose-3-C$^{14}$, was allowed to stand in a stoppered flask under nitrogen at 25° for 20 days. Sodium ions and neutral compounds then were separated from acidic products by passing the solution over appropriate columns of Amberlite IR-100[18] and Duolite A-4.[19] Acidic products were displaced from the Duolite A-4 column with 1 *N* sodium hydroxide, and the effluent was freed of sodium ions by passage over Amberlite IR-100. After titration with sodium hydroxide, the solution was concentrated to provide the mixed sodium salts.

To a solution of 10.2 g. of D-glycerose-3-C$^{14}$ in 125 ml. of water was added 5 g. of calcium hydroxide. The mixture was maintained under nitrogen in a stoppered flask at 25° for 5 months, and the sodium salts of the acidic products then were prepared as described above.

**Separation of Acetic, Formic and Lactic Acids.**—Aliquots of the mixed sodium salts, amounting to 20% of the totals, from the two reaction mixtures were chromatographed on silicic acid as described previously.[8] Efficient separations of pure acetic, formic and lactic acids were effected, with

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN THE ACIDIC PRODUCTS FROM THE REACTION OF D-GLYCEROSE-3-C$^{14}$ WITH ALKALI

| Sample | Carbon atoms | Radioactivity, cts./min./mM × 10$^{-2}$ |
|---|---|---|
| **Sodium Hydroxide** | | |
| Lactic acid | | |
| 2-(α-Hydroxyethyl)-benzimidazole | 1, 2, 3 | 181 |
| Benzimidazole-2-carboxylic acid·2H$_2$O | 1, 2 | 80 |
| Benzimidazole | 1 | 80 |
| Acetic acid | | |
| 2-Methylbenzimidazole | 1, 2 | 88 |
| Benzimidazole | 1 | 4.4 |
| Formic acid | | |
| Benzimidazole | ... | 92 |
| **Calcium Hydroxide** | | |
| Lactic acid | | |
| 2-(α-Hydroxyethyl)-benzimidazole | 1, 2, 3 | 178 |
| Benzimidazole-2-carboxylic acid·2H$_2$O | 1, 2 | 88 |
| Benzimidazole | 1 | 65 |
| Acetic acid | | |
| 2-Methylbenzimidazole | 1, 2 | 104 |
| Benzimidazole | 1 | 9.9 |
| Formic acid | | |
| Benzimidazole | ... | 69 |

(15) Obtained from the National Bureau of Standards through the courtesy of Dr. Horace S. Isbell.

(16) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 463 (1939); THIS JOURNAL, **61**, 761 (1939).

(17) All radioassays were performed directly on thin samples (75–100 μg./sq. cm.) as described previously.[8]

(18) A product of Rohm and Haas Co., Philadelphia, Pa.

(19) A product of Chemical Process Co., Redwood City, Calif.

peak volumes essentially the same as those observed previously.[8] The observed, total yields of acids, corrected for an established slight loss on the silicic acid columns, were 28 meq. of lactic acid, 1.6 meq. of formic acid, 0.8 meq. of acetic acid and 6.0 meq. (by difference) of other acids from the reaction with sodium hydroxide; and 12 meq. of lactic acid, 5.8 meq. of formic acid, 1.5 meq. of acetic acid and 20 meq. (by difference) of other acids from the reaction with calcium hydroxide.

**Radioassay of Derivatives.**—The separated samples of formic acid were converted to benzimidazole; those of acetic acid to 2-methylbenzimidazole and benzimidazole; and those of lactic acid to 2-(α-hydroxyethyl)-benzimidazole, benzimidazole-2-carboxylic acid and benzimidazole as described previously.[8,10] All purified samples for radioassay showed melting points in agreement with reported values. Radioassay data[17] for the various derivatives are shown in Table I.

**"α"-D-Glucosaccharinic Lactone.**—After elution from the silicic acid column of the acetic, formic and lactic acids produced in the reaction with calcium hydroxide (20% aliquot) with 1-butanol–chloroform,[8] the column was further developed with benzene–methanol (8:1, v./v.). A fraction of acidic material, containing sulfuric acid from the preconditioning of the column and organic acids, was obtained at a peak volume of 175 ml. Sulfate was precipitated from this fraction with barium hydroxide and barium ions then were removed from the filtered solution by ion-exchange. Concentration yielded a light-colored sirup which was seeded with "α"-D-glucosaccharinic lactone. After several months, the crystals that had formed were separated on a porous clay plate and recrystallized from water. The product (*ca.* 30 mg.) showed the correct properties for "α"-D-glucosaccharinic lactone: m.p. 160–162°, $[\alpha]^{25}D$ 92° in water, *c* 0.5; quinine salt,[20] m.p. 140–142° and $[\alpha]^{25}D$ −114° in water, *c* 0.5. Radioactivity of the lactone was approximately 4 × $10^5$ cts./min./mM.

(20) Nef[5] reports m.p. 152° for this salt. H. Kiliani and P. Loeffler, *Ber.*, **37**, 1196 (1904), record m.p. 141–142°, and H. Kiliani, P. Loeffler and O. Matthes, *ibid.*, **40**, 2999 (1907), record $[\alpha]D$ −102.6° in water. Prepared in this Laboratory from known "α"-D-glucosaccharinic lactone (obtained by the alkaline isomerization of D-mannose[8]) and recrystallized several times from water–acetone, the salt showed m.p. 141–142° and $[\alpha]^{28}D$ −115° in water, *c* 0.6.

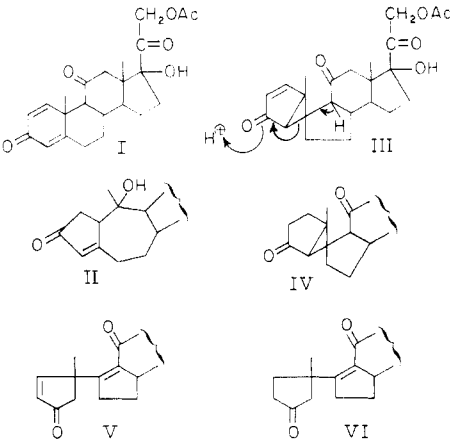
ST. LOUIS 5, MISSOURI

# COMMUNICATIONS TO THE EDITOR

## THE PHOTOCHEMISTRY OF PREDNISONE ACETATE IN NEUTRAL SOLUTION

*Sir:*

We recently reported[1] that the medicinally important[2] prednisone acetate (I) was converted on irradiation in refluxing aqueous acetic acid into II. Such a rearrangement reaction has been well exemplified.[3] In neutral solution analogy with the photochemistry of santonin[4] would suggest that products of a different type would be formed. We now wish to report that irradiation of I with ultraviolet light in ethanol solution affords lumiprednisone acetate (III), m.p. 224–226°, $[\alpha]D$ −84° (*c* 0.80; all rotations in $CHCl_3$), $\lambda\lambda_{max}$ 218 and 265 mμ (ε 5,900 and 2,300, respectively; all ε in EtOH), infrared max. (all max. in $CHCl_3$) at 1735 (21 acetate), 1708 (11 and 20 ketones), 1690 (conjugated ketone) and 1575 (conjugated C=C) cm.$^{-1}$; calcd. for $C_{23}H_{28}O_6$: C, 69.0; H, 7.05; 3 C–Me (1 from the 21 OAc), 11.3. Found: C, 68.95; H, 7.05; C–Me, 9.5%. The spectra are typical of an umbellulone.[4,5] Palladized charcoal hydrogenation of III gives a saturated dihydro-derivative IV, m.p. 200–203°, $[\alpha]D$ +95° (*c* 1.15), $\lambda_{max}$ 207 mμ (ε 8,100); expected infrared max.; calcd. for $C_{23}H_{30}O_6$: C, 68.6; H, 7.5. Found: C, 68.8; H, 7.65. The ultraviolet spectrum coupled with stability to ozone show the presence of conjugated ketone and cyclopropane groups.[4,5] Treatment of III with 0.2% $HClO_4$ in acetic acid at 85° for 20 minutes affords an isomer V, m.p. 202–204°, $[\alpha]D$ −103° (*c* 0.50), $\lambda_{max}$ 241 mμ (ε 10,600), expected infrared max.; found: C, 68.8; H, 6.85; C–Me, 11.9%. Hydrogenation over palladized charcoal gives a dihydro-derivative VI, m.p. 192–195°, $[\alpha]D$ +127° (*c* 0.50), $\lambda_{max}$ 251 mμ (ε 8,700), expected infrared max.; found: C, 68.6; H, 7.35; also obtained from IV by isomerization over alumina (Brockmann[6] Grade III). The ultraviolet subtraction curve of VI from V gave $\lambda_{max}$ 219 mμ (ε 7,300) in a good agreement[4,7] with that expected

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(2) H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, THIS JOURNAL, **77**, 4781 (1955); A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *ibid.*, **77**, 4184 (1955)

(3) D. H. R. Barton, P. de Mayo and M. Shafiq, *J. Chem. Soc.*, 929 (1957); D. H. R. Barton and J. E. D. Levisalles, unpublished observations.

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(6) H. Brockmann and H. Schodder, *Ber.*, **74**, 73 (1941).

(7) R. B. Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 76 (1942); A. E. Gillam and T. F. West, *J. Chem. Soc.*, 486 (1942).