Under the conditions of the above spot test, up to 40–50 γ of a steroid with a dihydroxyacetone side-chain per 1 cm. width of chromatogram will be destroyed in the allotted time. Exposure to higher temperatures than 100–110° or concentrations of alkali greater than 0.1 *N* will destroy some of the α-ketol group. The appearance of a light yellow-pink color after the test has been executed, as compared to the vivid red on the first strip, should be taken as a positive test for the dihydroxyacetone group.

This test has been used successfully on chromatograms in differentiating the side-chains of cortisone, hydrocortisone, substance S of Reichstein, and substance D of Reichstein from corticosterone, desoxycorticosterone and allopregnane-3β,21-diol-20-one.

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Synthesis of Ethyl Ethoxybromoacetate and Ethyl Ethoxycyanoacetate[1]

BY AARON BENDICH AND GRACE C. CLEMENTS

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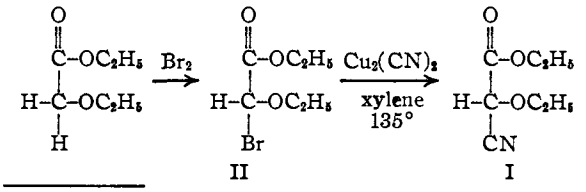
Ethyl ethoxycyanoacetate (I) was required for the preparation of substituted pyrimidines[2] containing the ethoxy group at position 5. The carbethoxylation reaction of aliphatic nitriles with diethyl carbonate described by Wallingford[3] was attempted on ethoxyacetonitrile, but the desired ester I was obtained apparently together with its ethylated derivative ethyl α-ethoxy-α-ethylcyanoacetate. It has been pointed out previously[4] that in such carbethoxylation reactions, simultaneous alkylation by the alkyl carbonate often occurs.

The alternate route, described below, affords the ester I in good yield. For this method, ethyl ethoxybromoacetate (II) was prepared by direct bromination of ethyl ethoxyacetate in carbon tetrachloride solution. The bromo ester II possesses an extremely reactive bromo atom which is easily hydrolyzed on brief contact with cold water. It also readily produces iodine upon treatment with aqueous potassium iodide.

Several unsuccessful attempts were made to convert the bromo ester II to the cyano ester I upon treatment with either potassium or cuprous cyanide in methanol, ethanol, diethyl ether or benzene. A 55% yield of the cyano ester I was obtained upon refluxing a suspension of cuprous cyanide in a xylene solution of II.



Experimental

Ethyl Ethoxybromoacetate.—Ethyl ethoxyacetate (66 g., 0.5 mole) was dissolved in 65 ml. of carbon tetrachloride. The solution was stirred and kept refluxing while 80 g. (0.5 mole) of bromine was added at a rate such as to prevent the presence of excess bromine. The addition of bromine required about 3 hours. The HBr that had formed was removed by aeration and the residue was fractionated *in vacuo*. The fraction boiling 82–93° at about 10 mm. was redistilled; b.p. 94–95° (15 mm.) (197° at 750 mm.). The yield was 79 g. (75%).

Anal. Calcd. for $C_6H_{11}O_3Br$: C, 34.14; H, 5.25; Br, 37.86. Found: C, 33.93; H, 5.61; Br, 37.63.

Ethyl Ethoxycyanoacetate.—Ethyl ethoxybromoacetate (15 g., 0.071 mole) was dissolved in an equal volume of xylene (b.p. 135°) and 13.6 g. of *cuprous cyanide* (0.076 mole of $Cu_2(CN)_2$ was added. The mixture was stirred vigorously and was refluxed for 5 hours at the end of which time it no longer released iodine upon treatment with aqueous potassium iodide. The insoluble salts were removed and the fluid was distilled *in vacuo*. The fraction boiling at 94–100° at 11 mm. was collected; yield 6.2 g. (55%). It was refractionated: b.p. 95.0–96.5° (11 mm.), 217–218° (750 mm.).

Anal. Calcd. for $C_7H_{11}O_3N$: C, 53.48; H, 7.05; N, 8.91; total OC_2H_5, 57.33. Found: C, 53.46; H, 7.05; N, 8.81; total OC_2H_5, 57.22.

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(1) This investigation was supported by grants from the National Cancer Institute, National Institutes of Health, United States Public Health Service, and from the Atomic Energy Commission, Contract No. AT(30-1)-910.

(2) A. Bendich, *Trans. N. Y. Acad. Sciences, Ser. II*, **15**, 58 (1952).

(3) V. H. Wallingford, D. M. Jones and A. H. Homeyer, THIS JOURNAL, **64**, 576 (1942).

(4) V. H. Wallingford, A. H. Homeyer and D. M. Jones, *ibid.*, **63**, 2056 (1941).

The Metabolic Fate of Imidazoleacetic($C^{14}OOH$) Acid in the Rat

BY L. P. BOUTHILLIER AND GILLES LÉVEILLÉ

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It has been shown conclusively that imidazoleacetic acid is a product of oxidation of histamine in the intact rat.[1–3] However, the metabolism of imidazoleacetic acid is as yet unknown and the question arises whether this substance can be broken down or not *in vivo*. In this paper, we wish to report the results of experiments which provide evidence that this metabolite does not undergo oxidation in the rat tissues. Single doses of imidazoleacetic ($C^{14}OOH$) acid were administered intraperitoneally to three rats. The respiratory carbon dioxide and urine were collected for a period of 24 hours and the radioactivity of each was determined. Our experimental data, summarized in Table I, show that nearly 90% of the injected radiocarbon was recovered as imidazoleacetic acid in the urine samples, through the use of the isotope dilution technique. However, no radioactivity could be measured in the expired carbon dioxide. Employing the ascending method, one-dimensional paper chromatograms of urine samples were prepared in *n*-butanol–glacial acetic acid–

(1) A. H. Mehler, H. Tabor and H. Bauer, *J. Biol. Chem.*, **197**, 475 (1952).

(2) H. Tabor, A. H. Mehler and R. W. Schayer, *ibid.*, **200**, 605 (1953).

(3) L. P. Bouthillier and Moe Goldner, *Arch. Biochem. Biophys.*, In press.

TABLE I

	Rat 1	Rat 2	Rat 3
Weight of animal, g.	51	52	55
Amount of imidazoleacetic acid injected, mg.	15.6	20.9	16.2
Total radioactivity injected, c.p.m.	1.56×10^5	2.09×10^5	1.62×10^5
Radioactivity of expired CO_2	Nil	Nil	Nil
Total radioactivity of urine, c.p.m.	1.39×10^5	1.89×10^5	1.45×10^5
Total radioactivity of urinary imidazoleacetic acid, c.p.m.	1.41×10^5	1.81×10^5	1.42×10^5
Per cent. of injected radioactivity recovered in urine as imidazoleacetic acid	90.4	86.6	87.7

water as solvent system. The Pauly diazo reaction, carried out on a series of chromatograms, revealed the presence of only one imidazole compound; a single red-colored spot of R_F 0.34 appeared, corresponding to imidazoleacetic acid. Another series of chromatograms were cut into small segments and each segment was assayed for radioactivity. The average activity values, expressed in c.p.m. and corrected for background only, are presented in Fig. 1. A single radioactive peak was found which coincided with the imidazoleacetic acid spot.

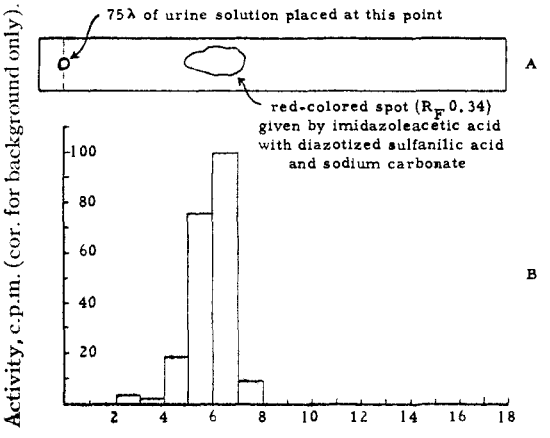

Fig. 1.—A, typical paper chromatogram of urine solution prepared with *n*-BuOH–AcOH–H_2O; B, radioactivity distribution on paper chromatogram of urine solution.

In the light of the data reported here, the conclusion is reached that imidazoleacetic acid is not metabolized to any measurable extent in the rat organism. In unpublished preliminary experiments carried out with ring-labeled imidazole-2-C^{14}-acetic acid, we have obtained results comparable to those given in this paper. We wish to draw attention to the report of Mehler, Tabor and Bauer[1] who "found that only one-third of a 246 μM dose (40 mg.) of imidazoleacetic acid hydrochloride, injected intraperitoneally into a 250-g. rat, was found in the urine." The discrepancy which exists between their result and ours is for the moment unexplainable.

We wish to report also that purified imidazoleacetic acid hydrate produces toxic effects when injected to 50-g. rats at levels of 5 mg. or higher. It induces irregular respiration and nervous disorders accompanied by an increased sensitivity to noise. The toxic manifestations lasted for one to three hours depending on the dose administered. No attempt was made to determine the lethal dose.

Experimental Part

Synthesis of Imidazoleacetic($C^{14}OOH$) Acid.—The method of Darby, Lewis and Totter[4] was employed for the preparation of hydroxymethylimidazole picrate. Regeneration of hydroxymethylimidazole hydrochloride from the picrate was made according to the method of Koessler and Hanke.[5] By reaction of hydroxymethylimidazole hydrochloride with phosphorus pentachloride,[6] chloromethylimidazole hydrochloride was obtained. Employing the method of Pyman,[6] 1 g. of the latter compound was made to react with 3 g. of $KC^{14}N$ (about 0.5 mc.) and the resulting nitrile was converted into ethyl imidazoleacetate and subsequently into imidazoleacetic($C^{14}OOH$) acid hydrochloride. Free imidazoleacetic acid was prepared by treatment of an aqueous solution of the hydrochloride with silver carbonate. The over-all yield in the synthesis, calculated on the basis of chloromethylimidazole hydrochloride employed, was about 18%. Pure crystals of imidazoleacetic acid monohydrate,[6] melting with decomposition at 217–218° (uncor.), were obtained by crystallization of the synthetic product in a mixture of water and acetone. Analysis for $C_5H_6O_2N_2$ (anhydrous form obtained after treatment in an Abderhalden apparatus): N, calculated 22.22%; found, 21.69%. The specific activity was measured with a windowless flow counter and calculated to be 1.0×10^4 counts-minute-mg.

Administration of Labeled Imidazoleacetic Acid to Rats and Collection of Exhaled CO_2 and Urine.—Known amounts of the radioactive material (Table I) were dissolved in 1-ml. portions of water and the *p*H of each preparation was adjusted to 7.4 with sodium hydroxide. The solutions were administered to Wistar strain rats by way of intraperitoneal injection and each animal was placed in a glass metabolism cage for a period of 24 hours. The animals were allowed to drink water during the experimental period. The respiratory CO_2 was absorbed by sodium hydroxide solution and precipitated as barium carbonate. The 24-hour urine, preserved with thymol, was collected and completed to a volume of 25 ml. with the washings and distilled water.

Isolation of Urinary Imidazoleacetic Acid by the Carrier Method.—Into 5-ml. portions of diluted urine was dissolved 100 mg. of imidazoleacetic acid hydrate and 15 ml. of acetone was then added. The solutions were kept in a refrigerator until imidazoleacetic acid hydrate precipitated out. The product was recrystallized a few more times to constant radioactivity. The various samples obtained melted with decomposition at 218–220°.

Radioactivity Measurements.—Known quantities of barium carbonate and imidazoleacetic acid samples were uniformly spread on aluminum cups (7.2 cm.^{2}) and small volumes (1 ml. or less) of the urine samples were evaporated to complete dryness on similar cups. The radioactivity of the contents of each cup was measured with a windowless flow counter and corrected for background and internal absorption.

Chromatography Assays of Urine.—Seventy-five microliters of the urine solutions were placed on each paper chromatogram (Whatman No. 1). The ascending method was employed using, as chromatographic solvent system, a mixture of *n*-butanol, 300 ml., glacial acetic acid, 60 ml., and water, 140 ml.[7] Then the chromatograms were sprayed with a freshly diazotized sulfanilic acid solution (0.45%) and treated with finely powdered sodium carbonate. The appearance of a red-colored spot is characteristic of imidazole compounds (Pauly diazo reaction).

Another series of chromatograms was prepared and cut into half-inch-square segments. The radioactivity of each paper segment was measured with a thin mica window Geiger tube and the values were corrected for background only.

Acknowledgments.—This investigation was supported in part by a grant from the National Research Council of Canada, Division of Medical Research, and by the "Fondation Rhéaume." The

(4) W. J. Darby, H. B. Lewis and J. R. Totter, THIS JOURNAL, **64**, 463 (1942).
(5) K. K. Koessler and M. T. Hanke, *ibid.*, **40**, 1716 (1918).
(6) F. L. Pyman, *J. Chem. Soc.*, **99**, 668 (1911).
(7) J. L. Anclair and J. B. Maltais, *Nature*, **170**, 1114 (1952).

authors are indebted also to the Scientific Research Bureau, Department of Trade and Commerce, Province of Quebec, for a studentship granted to one of us (G.L.).

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Aromatic Substitution at Ortho-Positions

BY R. D. BROWN

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The interpretation of aromatic substitution in benzene derivatives in terms of the simple LCAO molecular-orbital approximation has now attracted the attention of a number of authors.[1-8] Early attempts[1,2] were concerned only with a demonstration that the theory points to *o*-, *p*-substitution for some kinds of derivatives, and *m*-substitution for others, in general agreement with observation. Dewar[4] considered the more sophisticated problem of *o*:*p* ratios. His results indicated that only in cases where the benzene substituent is very electronegative would the *p*-position be *electronically* more activated than *o*-, with the inference that the observed preponderance of *p*-substitution is occasioned in part at least by steric factors.[9] Additional weight is lent to Dewar's results because he was able to account qualitatively for trends in the *o*:*p* ratio as the nature of the substituent is varied.

If however the benzene substituent is a conjugated hydrocarbon system, as in biphenyl and styrene, the uncertainty about the electronegativity parameter disappears and the molecular-orbital approximation provides an unequivocal prediction of the electronic activities. For biphenyl the *o*-positions are predicted[5] to be appreciably more reactive than the *p*-positions. In this case, then, the observed low *o*:*p* ratio must be ascribed to steric factors. This result is not peculiar to biphenyl; similar relative electronic reactivities of *o*- and *p*-positions are predicted for vinyl- and 1-butadienyl substituents, as shown in Table I, and indeed there seems little doubt that for any conjugated hydrocarbon system as substituent the MO approximation will ascribe a greater electronic reactivity to the *o*-position as has been suggested by Roberts and Streitwieser. The results of Table I strictly apply only for the completely planar molecules. It is known[10] that biphenyl is on the average non-planar in the vapor phase, and presumably also in solution, and this would effectively reduce the disparity in the *o*:*p* reactivities, but it will not affect the qualitative relationship.

TABLE I

ATOM LOCALIZATION ENERGIES[8] ($-\beta$)

	Biphenyl	Styrene	1-Phenyl-butadiene
Ortho	2.400	2.370	2.313
Para	2.447	2.424	2.377

The attribution of the observed superior reactivity of the *p*-position in biphenyl to steric effects rather than to a failure in the MO theory is supported by some further theoretical work of Dewar.[6] He has computed approximate atom localization energies for a considerable number of polycyclic aromatic hydrocarbons. In all cases where the position of substitution is known experimentally it coincides with the position which theory predicts to be electronically most reactive, with the single exception of triphenylene. But in this molecule the 1-position, which has a steric environment analogous to an *o*-position in biphenyl, is predicted to be the most active, while substitution is observed[11] to occur in the 2-position. In all other cases considered by Dewar the most reactive positions predicted by theory have steric environments much more favorable for chemical attack.

Since the only two molecules for which substitution occurs predominantly at positions other than those predicted by the MO localization theory are those for which the steric environments of the electronically most reactive positions are unfavorable for substitution, it seems justifiable to attribute the predominant *p*-substitution in biphenyl to steric hindrance of the *o*-positions.

Remick[12] has suggested, in terms of the qualitative electronic theory, that the superior reactivity of the *p*-position in biphenyl is to be anticipated solely on electronic grounds, using the principle that "the electromeric effect will operate more readily the more extended the conjugation becomes." However if this principle is applied to biphenyl in the same way in which Remick applies it[13] to explain the α-activation of pyrrole, furan and derivatives, it would point to *o*-activation; indeed Remick has noticed just this kind of difficulty[14] in applying the principle to other benzene derivatives.

Finally it should be observed that the data for styrene in Table I differ considerably from the figures recently published by Roberts and Streitwieser.[15] Both the absolute values of the localization energies and their relative values (their data indicate a greater *p*- than *o*-reactivity) are in error. The correct values, together with values of the free valences ($N_{max} = \sqrt{3}$) for comparison,[8] are listed in Table II. It will be observed that the free valences confirm the superior electronic reactivity of the *o*- as compared with the *p*-position.

(1) G. W. Wheland and L. Pauling, THIS JOURNAL, **57**, 2086 (1935).
(2) G. W. Wheland, *ibid.*, **64**, 900 (1942).
(3) C. Sandorfy, *Bull. soc. chim.*, **16**, 615 (1949).
(4) M. J. S. Dewar, *J. Chem. Soc.*, 463 (1949).
(5) R. D. Brown, *Experientia*, **6**, 376 (1950).
(6) M. J. S. Dewar, THIS JOURNAL, **74**, 3357 (1952).
(7) J. D. Roberts and A. Streitwieser, *ibid.*, **74**, 4723 (1952).
(8) For an account of the correlation of localization energies and free valences with chemical reactivities see R. D. Brown, *Quart. Rev.*, **6**, 63 (1952).
(9) More than this cannot be said because the appropriate electronegativity parameters for the various substituents are very uncertain.
(10) O. Bastiansen, *Acta Chem. Scand.*, **4**, 926 (1950).
(11) E. Clar, "Aromatische Kohlenwasserstoffe," Springer Verlag, Berlin, 1941, p. 104.
(12) A. E. Remick, "Electronic Interpretations of Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 103.
(13) Reference 12, pp. 104-105.
(14) Reference 12, p. 104.
(15) Dr. Roberts has kindly informed the author of his agreement with the present figures for styrene, and that the incorrect data in ref. 7 were due to errors in transcription.