THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 240, No. 3, March 1965
Printed in U.S.A.

Formation of Benzene and Toluene from Acetylene-^{14}C in the Avocado

EUGENE F. JANSEN AND JOAN M. WALLACE

From the Western Regional Research Laboratory, Albany 10, California*

(Received for publication, October 20, 1964)

In previous communications from this laboratory on the fate of ethylene in avocados, it was shown that approximately 12% of the radioactivity of metabolized ethylene-^{3}H appeared in the methyl group of toluene (1) whereas only approximately 0.2% of the radioactivity of metabolized ethylene-^{14}C appeared in toluene (2). On the other hand, benzene produced by the metabolism of ethylene-^{3}H and ethylene-^{14}C contained similar amounts of the total incorporated, 1% and 4%, respectively (2). These results suggest that, in the metabolism of ethylene, a considerable portion of the hydrogen is removed by some dehydrogenation process, and this hydrogen proceeds along a pathway different from that followed by the carbon, as is illustrated by the labeling of toluene. Nevertheless, part of the hydrogen and carbon of ethylene is metabolized along the same pathway, as illustrated by the labeling of benzene. This postulate suggests that acetylene may be metabolized more directly to some of the same metabolites as ethylene. Further, acetylene is known to produce some of the same physiological effects as ethylene, *e.g.* acetylene induces flowering in pineapple plants (3) and epinasty in plants (4). The concentration of acetylene required for the latter effect is 500 times greater than that with ethylene (4).

The present report is concerned with the metabolism of acetylene-^{14}C by avocado fruit. Under the same conditions used with labeled ethylene (1, 2), approximately 9 times as much acetylene-^{14}C as ethylene was metabolized in a 4-hour period. Two-fifths of the metabolized acetylene was found in the respiratory CO_2. Approximately three-fourths of the remainder in the fruit was volatile. The benzene and toluene were concentrated and derivatives were made therefrom as in the ethylene-^{14}C experiments (2). The benzene isolated from fruit treated with acetylene contained approximately 25 times as much ^{14}C as the benzene from ethylene-treated fruit, whereas the ^{14}C in toluene was the same for the two treatments.

EXPERIMENTAL PROCEDURE

The method of exposing mature, green avocado fruit (Haas variety) to radioactive acetylene was the same as that used with ethylene-^{3}H (1). The unmetabolized acetylene was collected in mercuric perchlorate solution (5) and the metabolic CO_2 as $BaCO_3$. The metabolites contained within the fruit were extracted with 70% alcohol as described previously (1, 2).

Acetylene-^{14}C (20 mc per mmole) was purchased from New England Nuclear Corporation,[1] who prepared the acetylene just prior to shipment and use.

The radioactivity contained in the CO_2 regenerated from the $BaCO_3$ obtained from the respiratory CO_2 was determined in a Dynacon electrometer manufactured by Nuclear-Chicago Corporation. In all other forms, the radioactivity of ^{14}C samples was determined by the liquid scintillation system in naphthalene-dioxane scintillation solution with a Tri-Carb spectrometer manufactured by Packard Instrument Company, Inc. Corrections for the quenching effect of the several derivatives of toluene and benzene were determined by internal standard procedure.

RESULTS

Amount of Acetylene Metabolized—The amount of acetylene-^{14}C metabolized by avocados as measured by the radioactivity in the aqueous ethanol extracts and in the respiratory CO_2 is shown in Table I. The total amount metabolized was $9\frac{1}{2}$ times greater than was ethylene at the same concentration and under the same conditions (2). The amount of acetylene-^{14}C converted to CO_2 was over 300 times greater than of ethylene (2). The $BaCO_3$ was reformed by conversion to CO_2 in a stream of N_2 and reprecipitation as $BaCO_3$ two successive times without altering its specific radioactivity, showing that the high radioactivity did indeed reside in the respiratory CO_2 rather than in an impurity. Acetylene is evidently more completely metabolized by avocados in 4 hours than is ethylene.

Volatile Metabolites—The major portion of the acetylene-^{14}C metabolites contained in the aqueous-alcohol extracts was found to be volatile. On evaporation of a portion of the extract in vacuum at a maximal temperature of 35° and collection of the condensate in a Dry Ice followed by a liquid nitrogen trap, approximately three-fourths of these metabolites were found in the condensate and one-fourth in the distillation residue. Three-fourths of the metabolites of ethylene-^{3}H (1) and only one-fourth of those of ethylene-^{14}C were volatile (2). The volatile metabolites from 2 liters of extract were collected and fractionally distilled in a 10-plate Fenske packed column in the same manner as were those from labeled ethylene (1, 2). The first distillation fraction (10.6 ml or 0.5% of the total) contained 60% of the volatile metabolites.

Benzene Derivatives—The amount of radioactivity contained in the benzene present in the first distillation fraction was deter-

* One of the laboratories of the Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

[1] Reference to a company or product name does not imply approval or recommendation of the product by the Department of Agriculture to the exclusion of others that may also be suitable.

Downloaded from www.jbc.org at UNIV OF CALIFORNIA RIVERS, on February 9, 2013

mined by transfer of the benzene to a nonpolar solvent, addition of unlabeled benzene, and the determination of radioactivity in the benzene derivatives, β-benzoylpropionic acid, benzoic acid, and p-phenylphenacylbenzoate. For this purpose, 3 ml of this fraction (1.64×10^6 c.p.m.), after dilution with 25 ml of H_2O, were extracted with 10 ml of cyclohexane. After separation of the cyclohexane extract, the aqueous phase was shaken successively with 10 and then 5 ml of 1,1,2,2-tetrachloroethane. The three extracts were combined and dried over anhydrous Na_2SO_4 and then diluted to 37 ml with tetrachloroethane. The cyclohexane-tetrachloroethane solution contained 1.17×10^6 c.p.m. or 71% of the radioactivity in the 3 ml of distillate taken.

To 18 ml of the cyclohexane-tetrachloroethane solution (5.69×10^5 c.p.m.) 1.0 ml of benzene was added, and the solution was subjected to the Friedel-Craft reaction with succinic anhydride by the procedure of Reinheimer and Taylor (6) as previously modified (2). The reaction mixture was steam-distilled. The steam distillate contained 42% of the radioactivity present in the original 18 ml of solution. The aqueous solution of β-benzoylpropionic acid was evaporated to dryness in order to remove the HCl; the derivative was dissolved in water and purified by adsorption on a Dowex 1-formate column and elution with 1 N formic acid (1). The elution peak corresponding to β-benzoylpropionic acid was divided into seven segments. Each segment was identical in specific radioactivity. After recrystallization from water, a yield of 740 mg of product (m.p. observed, 117–117.5°; reported, 116.5–117.5° (6)) was obtained. The specific radioactivity of the β-benzoylpropionic acid was 51,800 c.p.m. per mmole, which was unaltered by recrystallization from n-heptane (Table II). When allowance is made for the different amounts of the respective first distillation fractions used for the preparation of this derivative, the specific radioactivity of benzene arising from acetylene-^{14}C was 58 times as great as that from ethylene-^{14}C (2). When adjusted on the assumption of a 100% yield with respect to the added benzene in the Friedel-Craft reaction, the activity found in the β-benzoylpropionic acid represents approximately 30% of the radioactivity incorporated into the fruit, as compared with 3.8% for benzene arising from ethylene-^{14}C (2). Evidently benzene is formed more directly from acetylene-^{14}C than from ethylene-^{14}C.

That the radioactivity resided in the benzene nucleus of the β-benzoylpropionic acid was further confirmed by its oxidation to benzoic acid (2, 6) and the preparation therefrom of p-phenylphenacylbenzoate (2, 7). Both of these compounds possessed essentially the same specific radioactivity as the β-benzoylpropionic acid from which they were prepared (Table II).

Toluene Derivatives—The amount of radioactivity contained in the toluene present in the first distillation fraction was determined by procedures similar to those used with the benzene. To another 18 ml of the cyclohexane-tetrachloroethane solution (see above) was added 1.0 ml of toluene, and the solution was subjected to the Friedel-Craft's reaction in the same manner as was that containing added benzene. The steam distillate from the reaction mixture contained approximately 80% of the radioactivity present in the original 18 ml of solution. The aqueous solution of β-(p-methylbenzoyl)propionic acid was evaporated to dryness. The acid was dissolved in water and purified by adsorption on a Dowex 1-formate column and elution with 1 N formic acid (1). The elution peak corresponding to β-(p-methylbenzoyl)propionic acid was divided into seven segments. All of the material in the first and last two segments possessed a much higher specific radioactivity than that in the middle segments. The β-(p-methylbenzoyl)propionic acid (850 mg) contained in the middle segments was further purified by rechromatography with the same kind of column and eluent. In this case only the material in the first segments of the elution peak possessed a higher specific radioactivity than the remaining segments, which were constant. The β-(p-methylbenzoyl)propionic acid (490 mg) contained in the latter was pooled and recrystallized from

TABLE I

*Amount of acetylene-^{14}C metabolized by three avocados in 4 hours**

	Amount metabolized	
	Radioactivity†	Total fed
	μc	%
Incorporated into the fruit..	42	0.21
Respiratory CO_2...........	31	0.16

* The surrounding atmosphere contained 20 mc of radioactivity in 26 mg of acetylene which was present in a concentration of 2000 p.p.m.

† Corrected for counting efficiencies.

TABLE II

Specific radioactivities of β-benzoylpropionic acid, benzoic acid, and p-phenylphenacylbenzoate derived from benzene arising from acetylene-^{14}C

Compound and treatment	Radioactivity*
	c.p.m./mmole
β-Benzoylpropionic acid	
Recrystallized from H_2O†	51,800
Subsequent recrystallization from n-heptane	52,500
Benzoic acid	
Recrystallized from H_2O	52,000
p-Phenylphenacylbenzoate	
Recrystallized from 80% ethanol	50,900
Subsequently recrystallized from 80% ethanol	51,500

* Corrected for quenching.

† Subsequent to chromatographic purification; see text.

TABLE III

Specific radioactivity of toluene derivatives prepared from toluene arising from acetylene-^{14}C metabolism

Compound and treatment	Radioactivity*
	c.p.m./mmole
β-(p-Methylbenzoyl)propionic acid	
Recrystallized from H_2O†	110
Subsequently recrystallized from n-heptane	100
Again recrystallized from n-heptane	100
p-Toluic acid	
Recrystallized from H_2O	90
p-Phenylphenacyl-p-toluate	
Recrystallized from 80% ethanol	100
Subsequently recrystallized from 80% ethanol	90

* Corrected for quenching.

† Subsequent to chromatographic purification; see the text.

Downloaded from www.jbc.org at UNIV OF CALIFORNIA RIVERS, on February 9, 2013

water, yielding 470 mg of the pure acid (m.p., observed, 128.5–129°; reported, 127–128.5° (6)). The specific radioactivity of the derivative was only 110 c.p.m. per mmole, which was lowered slightly by recrystallization from *n*-heptane (Table III). When adjusted on the assumption of a 100% yield with respect to the added toluene in the Friedel-Craft reaction, the radioactivity found in the β-(*p*-methylbenzoyl)propionic acid represents only 0.06% of that incorporated into the fruit.

The radioactivity of the toluene was further confirmed by the oxidation of a portion of the β-(*p*-methylbenzoyl)propionic acid to *p*-toluic acid (1, 6) and the preparation therefrom of *p*-phenylphenacyl-*p*-toluate (1, 7). Both of these compounds possessed essentially the same radioactivity as the β-(*p*-methylbenzoyl)-propionic acid from which they were prepared (Table III).

Therefore, toluene is a metabolite of acetylene-^{14}C. However, the low radioactivity found in toluene suggests that it is a metabolite well along the pathway of acetylene metabolism. It is significant that the specific radioactivity of the toluene arising from acetylene was essentially identical to that found for toluene arising from ethylene in the avocado under identical conditions of exposure and specific radioactivity of the respective gases (2).

DISCUSSION

On the basis of the specific radioactivity found for the benzene derivatives and considering the amount of benzene found in extracts of avocado (1, 2), as well as the amount of benzene used presently for dilution purposes, the specific radioactivity of benzene arising from acetylene-^{14}C was 0.5% of that of the acetylene to which the avocados were exposed. This suggests that there is a reasonably short metabolic pathway between acetylene and benzene. Further, if the benzene were formed directly and exclusively from acetylene, the specific radioactivity of the benzene derivatives would have been considerably higher than the values observed, thus showing that benzene *per se* is present in green avocado fruit.

Acetylene produces some of the same physiological effects on plants and fruit as does ethylene, but the concentrations required for these effects are much greater for acetylene than for ethylene (3, 4). Yet avocados metabolize much more acetylene than ethylene, approximately 9 times more under the same conditions. Furthermore, the benzene arising from acetylene-^{14}C possesses a specific radioactivity almost 50 times greater than that from ethylene-^{14}C. The respiratory CO_2 from the former possessed a specific radioactivity 300 times as great as the latter. However, the toluene arising from both possesses essentially identical specific radioactivity. These results suggest that toluene arising from the carbon of either acetylene or ethylene is well along the metabolic pathway, particularly as compared with that arising from the hydrogen of ethylene (1).

Preliminary tests show that benzene and toluene are metabolized by avocado fruit. Their fate in the fruit and their possible effect on postharvest maturation are being studied.

SUMMARY

1. Acetylene-^{14}C (20 mc per mmole) in the surrounding atmosphere was metabolized by avocado fruit to the extent of approximately 0.5% in 4 hours. Approximately two-fifths of that metabolized was found in the respiratory CO_2, the remainder as metabolites in the fruit.

2. Approximately three-fourths of the metabolites in the fruit were volatile.

3. The benzene, as determined from derivatives prepared from it, was labeled in an amount corresponding to 30% of the metabolites contained in the fruit. The specific radioactivity of the benzene indicates a relatively short metabolic pathway between acetylene and benzene.

4. Toluene in the volatile metabolites was also labeled but represented only 0.06% of the metabolites contained in the fruit.

5. The significance of these findings as compared with those found with ethylene-^{14}C are discussed.

REFERENCES

1. Jansen, E. F., *J. Biol. Chem.*, **238**, 1552 (1963).
2. Jansen, E. F., *J. Biol. Chem.*, **239**, 1664 (1964).
3. Lewcock, H. K., *Queensland J. Agr. Sci.*, **48**, 532 (1937).
4. Crocker, W., Zimmerman, P. W., and Hitchcock, A. E., *Contribs. Boyce Thompson Inst.*, **4**, 177 (1932).
5. Young, R. E., Pratt, H. K., and Biale, J. B., *Anal. Chem.*, **24**, 551 (1952).
6. Reinheimer, J. D., and Taylor, S., *J. Org. Chem.*, **19**, 802 (1954).
7. Drake, N. L., and Bronitsky, J., *J. Am. Chem. Soc.*, **52**, 3715 (1930).

Downloaded from www.jbc.org at UNIV OF CALIFORNIA RIVERS, on February 9, 2013