The Journal of Biological Chemistry
Vol. 239, No. 5, May 1964
Printed in U.S.A.

Metabolism of Labeled Ethylene in the Avocado

II. BENZENE AND TOLUENE FROM ETHYLENE-^{14}C; BENZENE FROM ETHYLENE-^{3}H

Eugene F. Jansen

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

(Received for publication, November 15, 1963)

In a previous communication from this laboratory on the fate of ethylene in green avocados, it was shown that approximately 12% of the radioactivity of metabolized ethylene-^{3}H appears in the methyl group of toluene (1). Less than 0.6% of the metabolized tritium appeared in the benzenoid portion (1) of the toluene. The present report concerns the comparatively small incorporation of ^{14}C from labeled ethylene into toluene, which amounted to only about 0.2% compared with 12.5% for tritium, and with the incorporation of tritium and ^{14}C from labeled ethylene into benzene, where more nearly the same percentages of tritium and ^{14}C were found, namely, 1% and 4%, respectively.

MATERIALS AND METHODS

The method of exposing the mature green avocado fruit (Haas variety) to radioactive ethylene, the collection of the unmetabolized ethylene as the mercuric perchlorate complex (2) and of the metabolic CO_2 as $BaCO_3$, and the extraction of the metabolites from the fruit with 70% ethanol were described previously (1). Approximately 90% of the radioactivity incorporated into the fruit was found to be extractable with 70% ethanol.

Uniformly labeled ethylene-^{14}C was purchased from New England Nuclear Corporation, Boston.[1] To avoid polymerization of the highly radioactive ethylene (approximately 20 mc per mmole), the manufacturer prepared the ethylene just before shipment and sent it packed in Dry-Ice.

The radioactivity of ^{14}C in the form of CO_2 was determined in the Dynacon electrometer manufactured by Nuclear-Chicago Corporation. In all other forms, the radioactivities of ^{14}C samples were determined by the liquid scintillation system in naphthalene-dioxane scintillation solution, the Tri-Carb spectrometer manufactured by the Packard Instrument Company, Inc., being used. The quenching effect of the several benzene and toluene derivatives was determined by adding various amounts of unlabeled derivative to standard benzoic acid-^{14}C in the scintillation solution.

EXPERIMENTAL PROCEDURE AND RESULTS

Amount of Ethylene Metabolized—The amount of ethylene-^{14}C incorporated into the avocados as measured by the radioactivity in the aqueous ethanol extracts is shown in Table I. At the lower level (250 p.p.m.), 0.018% of the total amount in the surrounding atmosphere was taken up; at the higher level (2000 p.p.m.), 0.043%. The amounts incorporated were very similar to those found with ethylene-^{3}H (1).

Amount of Ethylene-^{14}C Converted to CO_2—Part of the metabolized ethylene-^{14}C was oxidized to CO_2. To establish this, portions of the $BaCO_3$ containing the metabolic CO_2 were treated with acid, and the radioactivity of the CO_2 liberated was determined. The results obtained are given in Table II. At the 250 p.p.m. level, a larger fraction of the incorporated ethylene was oxidized to CO_2 than at the 2000 p.p.m. level. The significance of this is not clear, but evidently the amount of ethylene converted to CO_2 is not proportional to the amount incorporated by the fruit as might have been expected.

Volatile Metabolites—As in the experiments with ethylene-^{3}H, a part of the metabolites of ethylene-^{14}C was found to be volatile. After evaporation of portions of the 70% alcoholic extracts in vacuum at a maximal temperature of 55°, it was demonstrated that one-fourth of the metabolites were volatile at both levels of ethylene-^{14}C (Table III) as compared with three-fourths in the case of ethylene-^{3}H (1).

In order to characterize the volatile metabolites, 2 liters of the more highly active extract (from fruit treated at 2000 p.p.m.) were evaporated as described, and the volatile material was collected in a Dry-Ice trap followed by a liquid nitrogen trap. The material in the liquid nitrogen trap was diluted with 380 ml of 70% ethanol and combined with that in the Dry-Ice trap. The total activity in both traps was 520,000 c.p.m. This solution was distilled, after being refluxed for 4 hours, in a 10-plate Fenske packed column with a ratio of reflux to takeoff of 100:5. The results obtained on the first five fractions are given in Table IV. The first fraction, corresponding to 0.4% of the total solution, contained 66% of the radioactive components. The subsequent four fractions contained 7.5% of the radioactive volatiles.

Characterization of Low Boiling Fraction—The ultraviolet absorption spectrum of the first fraction revealed the presence of approximately equal amounts of benzene and toluene. The amount of each present in this fraction was the same as that found in the corresponding ethylene-^{3}H fraction (1), namely, approximately 3 mg of each or approximately 3 mg of each per 200 g of fruit.

In order to ascertain whether the benzene and toluene were labeled with ^{14}C, the material contained in the first distillation fraction was transferred to nonpolar solvents and diluted with cold carriers, and several derivatives were prepared.

β-Benzoylpropionic Acid, Benzoic Acid, and p-Phenylphenacylbenzoate—The radioactive material contained in 5.0 ml (180,000 c.p.m.) of the first distillation fraction (Table IV), after dilution

* 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.

with 20 ml of H_2O, was extracted with 10 ml of cyclohexane. After separation of the cyclohexane extract, the aqueous phase was shaken successively with 5 and then 10 ml of 1,1,2,2-tetrachloroethane. The three extracts were combined and dried over anhydrous Na_2SO_4 and then diluted to 39 ml with tetrachloroethane. The cyclohexane-tetrachloroethane solution contained 146,000 c.p.m. or 81% of the radioactivity in the 5 ml of distillate taken. To 19 ml of the cyclohexane-tetrachloroethane solution (71,200 c.p.m.) was added 1.0 ml of benzene, and the solution was immediately subjected to the Friedel-Craft reaction with succinic anhydride by the procedure of Reinheimer and Taylor (3), except that the aluminum chloride was added in 15 ml of tetrachloroethane. The reaction mixture was steam-distilled. The cyclohexane-tetrachloroethane phase of the distillate was found to contain 35% of the radioactivity present in the original 19 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 2.5 N formic acid (1). The specific radioactivity of the material in the segments of the elution peak corresponding to the β-benzoylpropionic acid was determined. The material comprising the first segment possessed a specific radioactivity 4 times as great as that in the remaining four, in which the specific radioactivity of the β-benzoylpropionic acid was constant.

Table I

Amount of ethylene-^{14}C incorporated into three avocados in 4 hours

Amount fed		Amount incorporated	
Weight	Radioactivity	Radioactivity*	Per cent of total fed
mg	*mc*	*μc*	
3†	2	0.35	0.018
24‡	18	7.7	0.043

* Corrected for counting efficiency.
† The surrounding atmosphere contained 250 p.p.m.
‡ The surrounding atmosphere contained 2000 p.p.m.

Table II

Amount of ethylene-^{14}C converted to CO_2 in 4 hours

Concentration to which fruit was exposed	Amount converted	
	Radioactivity*	Per cent of total incorporated
p.p.m.	*μc*	
250	0.049	14
2000	0.090	1.5

* Corrected for counting efficiency on the basis of a 95% efficiency.

Table III

Volatile and nonvolatile metabolites of ethylene-^{14}C

Concentration to which fruit was exposed	Volatile	Nonvolatile
p.p.m.	%	%
250	24	76
2000	25	75

Table IV

First five distillation fractions of 70% ethanol solution of volatile metabolites of ethylene-^{14}C

Fraction	Reflux temperature	Volume	Radioactivity	Per cent of total
		ml	*c.p.m./ml*	
1	78.0°	9.6	36,000	66.0
2	78.1	10.5	1,300	2.5
3	78.1	10.5	630	1.3
4	78.1	10.5	410	0.8
5	78.2	89	170	2.9

Table V

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

Compound and treatment	Radioactivity*
	c.p.m./mmole
β-Benzoylpropionic acid	
Recrystallized from H_2O†	1820
Subsequently recrystallized from *n*-heptane	1790
Again recrystallized from *n*-heptane	1800
Benzoic acid	
Recrystallized from H_2O	1770
Subsequently recrystallized from H_2O	1780
p-Phenylphenacylbenzoate	
Recrystallized from 80% ethanol	1770

* Corrected for quenching, if any, of the individual compound.
† Subsequent to chromatographic purification; see the text.

Accordingly, only the β-benzoylpropionic acid in the last four fractions was used for further characterization. After recrystallization from water, a yield of 640 mg of product (m.p. observed, 117°; reported, 116.5–117.5° (3)) was obtained. The specific radioactivity of the β-benzoylpropionic acid was found to be 1820 c.p.m. per mmole, which remained essentially constant for two subsequent recrystallizations from *n*-heptane (Table V). 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 was 23% of the total in the sample used to prepare it and represents 3.8% of the total ethylene-^{14}C incorporated into the avocados. Hence, benzene is a metabolite of ethylene ^{14}C.

As further confirmation, a portion of the β-benzoylpropionic acid was oxidized to benzoic acid, and the *p*-phenylphenacyl ester was prepared so that a comparison of their specific activities might be made. For this purpose, 350 mg of the thrice recrystallized β-benzoylpropionic acid was oxidized to benzoic acid with alkaline hypochlorite (3). After one recrystallization from water, 180 mg of benzoic acid (m.p. observed, 121°; reported, 122° (4)) were obtained. The specific radioactivity of the benzoic acid was identical with that of the β-benzoylpropionic acid from which it was prepared and was unchanged by subsequent recrystallization from water (Table V). The *p*-phenylphenacyl ester was prepared from 75 mg of the twice recrystallized benzoic acid by the method of Drake and Bronitsky (5). The ester was recrystallized from 80% ethanol, yielding 133 mg (m.p. observed, 169°; reported, 167° (5)). The specific radioactivity of the ester was the same as that of the benzoic and β-benzoyl-

TABLE VI

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

Compound and treatment	Radioactivity*
	c.p.m./mmole
β-(*p*-Methylbenzoyl)propionic acid	
Recrystallized from H_2O†	92
Subsequently recrystallized from *n*-heptane	87
Again recrystallized from *n*-heptane	86
p-Toluic acid	
Recrystallized from H_2O	94
Subsequently recrystallized from H_2O	86
p-Phenylphenacyl-*p*-toluate	
Recrystallized from 80% ethanol	89

* Corrected for quenching, if any, of the individual compounds.
† Subsequent to chromatographic purification; see the text.

propionic acids. These results confirm the conclusion that benzene is a metabolite of ethylene-^{14}C.

β-(p-Methylbenzoyl)propionic Acid, p-Toluic Acid, and p-Phenylphenacyl-p-toluate—In order to obtain chemical evidence of the presence of toluene, another 19 ml of the above cyclohexane-tetrachloroethane solution from the first distillation fraction were diluted with 1.0 ml of toluene, and β-(*p*-methylbenzoyl)propionic acid was prepared therefrom by the Friedel-Craft procedure used above. The cyclohexane-tetrachloroethane phase of the steam distillate after the reaction contained 55% of the radioactivity of the starting solution. The β-(*p*-methylbenzoyl)propionic acid was chromatographically purified, as described for the β-benzoylpropionic acid. Only the center of the elution peak comprising β-(*p*-methylbenzoyl)propionic acid was used for further study. After recrystallization of the derivative from water, a yield of 950 mg was obtained (m.p. observed, 129; reported, 127–128.5° (3)). The specific radioactivity of the β-(*p*-methylbenzoyl)propionic acid was 92 c.p.m. per mmole and was essentially unchanged by subsequent recrystallizations from *n*-heptane (Table VI). When adjusted on the assumption of a 100% yield from the added toluene in the Friedel-Craft reaction, the specific radioactivity of the β-(*p*-methylbenzoyl)propionic acid represents only 0.2% of the total ethylene-^{14}C incorporated into the avocados.

As further evidence that toluene is a relatively minor metabolite of ethylene-^{14}C, the β-(*p*-methylbenzoyl)propionic acid was oxidized to *p*-toluic acid, and its *p*-phenylphenacyl ester was prepared in order to compare their specific radioactivities with that of the parent compound. The oxidation of 600 mg of β-(*p*-methylbenzoyl)propionic acid to *p*-toluic acid by alkaline sodium hypochlorite (3) resulted in a yield of 330 mg of product once recrystallized from water (m.p. observed, 182°; reported, 181° (4)). The specific radioactivity of the *p*-toluic acid, which was changed little by recrystallization, was practically identical with that of the β-(*p*-methylbenzoyl)propionic acid from which it was prepared. The *p*-phenylphenacyl ester of the twice recrystallized *p*-toluic acid (150 mg) was prepared (5). After recrystallization from 80% ethanol, the yield was 250 mg of the ester (m.p. observed, 165°; reported, 165° (5)), which also possessed essentially the same specific radioactivity as the β-(*p*-methylbenzoyl)propionic acid (Table VI). Hence, toluene is indeed a relatively minor metabolite of ethylene-^{14}C.

Benzene from Ethylene-^3H—The β-benzoylpropionic acid which had been prepared from the first distillation fraction of the volatile metabolites of ethylene-^3H, and which had been previously purified chromatographically (1), was further purified. Derivatives were prepared in order to ascertain whether benzene is also a metabolite of ethylene-^3H. After the acid was recrystallized from water, it possessed a specific radioactivity of 500 c.p.m. per mmole. Subsequent recrystallizations from *n*-heptane failed to alter this specific activity. A portion of the β-benzoylpropionic acid was oxidized to benzoic acid, and the *p*-phenylphenacylbenzoate was prepared therefrom by the procedures above. The ester was recrystallized twice from 80% ethanol, acid-washed charcoal being used for decolorizing. The ester melted at 169.5–170° and had a specific radioactivity of 500 c.p.m. per mmole. Hence, benzene is also a metabolite of ethylene-^3H and represents approximately 1% of the total radioactivity incorporated into the avocado fruit.

DISCUSSION

As observed in the study of the metabolism of ethylene-^3H (1), the first distillation fraction of volatile metabolites contained substances other than benzene and toluene, since approximately 35% of the radioactive substances did not react when subjected to the Friedel-Craft procedure. The identity of these other volatile metabolites is currently being sought.

Definitely, part of the metabolized ethylene is oxidized to CO_2. These results appear to contradict those of Buhler, Hansen, and Wang (6), who found no radioactivity in the respiratory CO_2 of either pears or avocados which had been exposed to radioactive ethylene. However, the high specific activity of the ethylene used in the present work, many times greater than that used by Buhler *et al.* (6), made it possible to detect labeled CO_2.

Toluene produced by the metabolism of ethylene-^3H contained 12.5% of the total radioactivity incorporated by the ethylene metabolism (1). Further, approximately 95% of the radioactivity of the toluene was located in its methyl group. On the other hand, the toluene produced by the metabolism of ethylene-^{14}C contained only 0.2% of total radioactivity incorporated. However, the benzene produced by the metabolism of ethylene-^3H and ethylene-^{14}C contained 1 and 4% of the respective quantities incorporated. These amounts are reasonably similar. Hence, as previously postulated (1), these results suggest that in the metabolism of ethylene by the avocado, a considerable portion of the hydrogen is removed from the ethylene by some dehydrogenation process, and this hydrogen proceeds along a pathway different from that followed by the carbon. Nevertheless part of the hydrogen and carbon of ethylene are metabolized along the same pathway as is illustrated by the labeling of benzene. This postulate suggests that acetylene may be metabolized more rapidly and may yield the same metabolites as ethylene. Accordingly, the metabolism of labeled acetylene is currently under investigation.

SUMMARY

1. Ethylene-^{14}C (20 mc per mmole) at the levels of 250 and 2000 p.p.m. was incorporated into metabolic components of avocado fruit from the surrounding atmosphere in 4 hours in the amounts of 0.018 and 0.043%, respectively, as measured by the radioactivity of 70% ethanol extracts of the fruit. Such extracts contained over 90% of the metabolized ethylene-^{14}C (other than CO_2).

2. Small amounts of ethylene-^{14}C were converted to CO_2.

3. Approximately one-fourth of the radioactive metabolites found in the 70% ethanol extracts were volatile. The volatile metabolites were concentrated by fractional distillation, resulting in a concentration of 66% of the volatile metabolites in the first 0.4% of the distillate. Ultraviolet absorption spectra of this fraction revealed the presence of both benzene and toluene.

4. Benzene arising from ethylene-^{14}C was shown to be labeled by transferring the material contained in the first distillation fraction to nonpolar solvents, dilution with benzene, and the preparation therefrom of β-benzoylpropionic acid, benzoic acid, and *p*-phenylphenacylbenzoate. All of these derivatives on purification possessed the same specific radioactivity. The amount of radioactivity found in these derivatives corresponded to 3.8% of the total radioactivity incorporated by the ethylene-^{14}C metabolism.

5. Toluene produced by the metabolism of ethylene-^{14}C was also shown to be labeled as demonstrated by a similar series of reactions. The amount of radioactivity found corresponded to 0.2% of that incorporated during the metabolism.

6. Benzene arising from the metabolism of ethylene-^{3}H was found to be labeled as demonstrated by means of the same reaction series. The amount of radioactivity found approximated 1% of that incorporated during the ethylene-^{3}H metabolism.

Acknowledgments—The author is indebted to Mr. Glen F. Bailey for the ultraviolet absorption spectra, and to Dr. A. K. Balls for his valuable suggestions.

REFERENCES

1. Jansen, E. F., *J. Biol. Chem.*, **238,** 1552 (1963).
2. Young, R. E., Pratt, H. K., and Biale, J. B., *Anal. Chem.*, **24,** 551 (1952).
3. Reinheimer, J. D., and Taylor, S., *J. Org. Chem.*, **19,** 802 (1954).
4. Heilbron, I., and Bunbury, H. M., *Dictionary of organic compounds, Vol. IV*, Eyre and Spottiswoode, London, 1953.
5. Drake, N. L., and Bronitsky, J., *J. Am. Chem. Soc.*, **52,** 3715 (1930).
6. Buhler, D. R., Hansen, E., and Wang, C. H., *Nature*, **179,** 48 (1957).