Short Communication

Metabolism of Carbon-14-Labeled Benzene and Toluene in Avocado Fruit

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The ubiquitous occurrence of hydrocarbons including many aromatics in plants has been the subject of an extensive, careful review (2). However, the origin and metabolism of the aromatic hydrocarbons in plant tissue has received little attention. The intriguing possible biogenesis of aromatics from the prevalent polyacetylenes in plants has been suggested (7).

Previous reports from our laboratory demonstrated that benzene and toluene occur *per se* in green avocado fruit (3, 4, 5). A study of the metabolism of ethylene in green, mature avocado fruit demonstrated the conversion of ethylene-^{3}H to benzene-^{3}H and toluene-^{3}H and the conversion of ethylene-^{14}C to benzene-^{14}C and toluene-^{14}C in this fruit (3, 4). Approximately one-eighth of the total incorporated radioactivity of metabolized ethylene-^{3}H was converted to toluene-^{3}H with approximately 95 % of the radioactivity of the toluene located in the methyl group (3). The metabolism of ethylene-^{14}C results in a much smaller amount of label into toluene (4). The incorporation of tritium and ^{14}C from labeled ethylene into benzene is 1 % and 4 %, respectively (4). Compared to ethylene, acetylene is a better precursor of benzene but not toluene (5). Therefore, a study of the metabolism of ^{14}C-labeled benzene and toluene by this fruit was undertaken. It has now been found that these 2 aromatic hydrocarbons are metabolized by avocado fruit, toluene more extensively than benzene, to produce volatile and nonvolatile metabolites of as yet unknown nature. Both hydrocarbons are metabolized to a small but significant extent to CO_2. Physiologically, both benzene and toluene vapors produce changes in ripening avocado fruit. However, the level of these hydrocarbons which produce a visual effect was found to be several hundred times that used in the present tracer experiments (unpublished results).

The method of exposing avocado fruit to ^{14}C-labeled benzene or toluene (from New England Nuclear Corporation)[1] was the same as that used with labeled ethylene (3). Fuerte variety avocado fruit was used in these experiments. It was exposed to the vapor of the hydrocarbons which were circulated in the container by the method previously described (3). Three mature preclimacteric fruit were used in each experiment. After the incubation, CO_2-free air was passed through the container; the unmetabolized hydrocarbon was collected in a cold trap consisting of the respective unlabeled hydrocarbon and the CO_2 was collected in $Ba(OH)_2$ solution. The fruit was ground and extracted with aqueous ethanol as described previously (3). Two experiments were carried out with each ^{14}C-labeled hydrocarbon with an interval of approximately 1 year between experiments. The radioactivity of ^{14}C in the form of CO_2 was determined in a Dynacon electrometer (Nuclear-Chicago Corporation). The radioactivity of other samples was determined in the liquid scintillation system described by Butler (1), using a Tri-Carb liquid scintillation spectrometer (Packard Instrument Company, Incorporated). As a control, heated avocado fruit was exposed to labeled hydrocarbons. Criteria for inactivation was to heat the fruit sufficiently to inactivate polyphenoloxidase as determined by absence of darkening of a longitudinal cut surface on application of 5 % catechol in aqueous ethanol. Autoclaving at 15 psi for 5 min was required for this inactivation.

The amounts of the labeled hydrocarbons taken up from the vapor phase by the fruit used in the several experiments are given in table I. With autoclaved fruit 15 % of benzene-^{14}C and 21 % of toluene-^{14}C were taken up. It was later shown that this uptake was simply solution of the hydrocarbons in the heated fruit. Small but significant amounts of the hydrocarbons were metabolized to $^{14}CO_2$. The amounts are given in table I. With heated fruit considerably less than one-tenth as much radioactivity was associated with the $^{14}CO_2$.

Vacuum distillation of aliquots of the aqueous ethanol extracts followed by resolution of the resulting residue in 70 % ethanol demonstrated that small amounts of benzene-^{14}C but considerable amounts of toluene-^{14}C had been metabolized to nonvolatile metabolites (table I). With heated fruit consider-

[1] Reference to a company or product name does not imply endorsement by the United States Department of Agriculture to the exclusion of others which may also be suitable.

Table I. *Uptake*[1] *and Metabolism of Benzene-^{14}C and Toluene ^{14}C in Avocado*

Labeled substrate	Amount fed		Uptake[3]	Substrate fed converted to $^{14}CO_2$	Distribution of label Percent of uptake volatile	Percent of uptake nonvolatile	Ion exchange separation of nonvolatile metabolites. Percent of nonvolatile radioactivity in Fat	Basic	Acidic	Neutral
	mg	*μc*	%	%						
Benzene	7.8	100	25	0.007	98	1.7	4	15	24	57
Benzene	6.4	100	28	0.004	98	2.6	...	...	...	...
Toluene[2]	5.1	100	36	0.007	73	27	3	8	26	63
Toluene	5.7	100	32	0.01	70	30	...	...	...	...

[1] Exposure time of 4 hr.
[2] Ring labeled.
[3] As measured by the radioactivity of the 70 % ethanol extracts.

ably less than one-tenth as much radioactivity was found in the nonvolatile fraction with the respective hydrocarbons.

It might be expected that unaltered hydrocarbon would account for the radioactivity contained in the volatile fraction. However, this was shown not to be the case. A portion of the aqueous ethanol extract from the first of the benzene experiments was vacuum distilled and the condensate collected in a dry-ice trap backed by a liquid nitrogen trap. The condensates were pooled and subjected to fractional atmospheric distillation by the method used previously, where it was shown that benzene and toluene were contained in the first distillation fraction (3, 4). Radioactive substances were contained in all the distillation fractions as shown in table II. Hence, volatile compounds other than benzene were contained in the condensates. It is of particular interest that volatile radioactive substances distilled after the ethanol (fractions 9, 10, and 11). Hence, substances other than benzene are contained in the condensates. A similar distribution of label was obtained on distillation of the condensates from a toluene-^{14}C experiment.

The nonvolatile metabolites from both hydrocarbons were separated into classes of compounds by their solution in water, separation of the fat therefrom, and passage of the aqueous solution through ion exchange columns. The results are given in table I. That adsorbed on Dowex-50-H^+ form and elutable with ammonia is designated "basic," that subsequently adsorbed on Dowex-1-formate form and elutable with formic acid is designated "acid," and that which passed through the latter ion exchange column is designated as "neutral." It is apparent that both hydrocarbons are metabolized to a series of compounds and that the distribution between classes of compounds is similar for both. This distribution of nonvolatile metabolites between classes of compounds is very similar to that observed in the metabolism of both ethylene-^3H and ethylene-^{14}C (6).

These results demonstrate that in avocados the relatively inert hydrocarbons, benzene and toluene, are metabolized to a series of compounds, toluene to a greater extent than benzene. Both are metabolized to a small but significant extent to CO_2.

Table II. *Fractional Distillation of Volatile Substances From Benzene-^{14}C Metabolism*

A 250-ml portion of condensate was distilled.

Fraction	Reflux temp	Volume	Radioactivity	Part of total
No.	*deg*	*ml*	*cpm/ml*	%
1	78.0	10.3	58400	51.50
2	78.0	8.2	6760	4.75
3	78.0	10.0	4200	3.60
4	78.0	10.2	2670	2.31
5	78.0	10.2	1470	1.26
6	78.0	40.2	1400	4.82
7	78.0	90.5	1000	7.88
8	78.0	4.4	3530	1.32
9	80.0	2.0	18700	3.20
10	94.0	2.4	26500	5.43
11	97.0	4.0	1650	0.56
12	98.0	3.5	1400	0.42
13	98.0	8.2	1020	0.72
14	98.0	5.7	200	0.09
15[1]	...	35.0	180	0.54

[1] Material remaining in distillation flask.

Literature Cited

1. Butler, F. E. 1961. Determination of tritium in water and urine. Liquid scintillation counting and rate-of-drift determination. Anal. Chem. 33: 409–14.
2. Gerarde, H. W. and D. F. Gerarde. 1961, 1962. The ubiquitous hydrocarbons. Assoc. Food Drug Officials U.S. 25, 161–172 and 26, 3–24 and 65–77.
3. Jansen, E. F. 1963. Metabolism of labeled ethylene in the avocado. Appearance of tritium in the methyl group of toluene. J. Biol. Chem. 238: 1552–55.
4. Jansen, E. F. 1964. Metabolism of labeled ethylene in the avocado. II. Benzene and toluene from ethylene-^{14}C; benzene from ethylene-^3H. J. Biol. Chem. 239: 1664–67.
5. Jansen, E. F. and J. M. Wallace. 1965. Formation of benzene and toluene from acetylene-^{14}C in the avocado. J. Biol. Chem. 240: 1042–44.
6. Jansen, E. F. 1965. Ethylene and polyacetylenes. In: Plant Biochemistry. J. Bonner and J. E. Varner, eds. Academic Press, New York. 641–64.
7. Sörensen, J. S. and N. A. Sörensen. 1958. Studies related to naturally occurring acetylene compounds. XXII. Correctional studies on the constitution of polyacetylenes of some annual *Coreopsis* species. Acta Chem. Scand. 12: 756–64.