Carbon-14 in a Biological Environment. I. Annual Variation of Carbon-14 Concentration in Thymol

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Since 1954, the concentration level of C-14 in the atmosphere has remarkably increased as a result of the artificial production of C-14 by the successive nuclear testings; this tendency was confirmed by many investigators[1].

Recently, two excellent methods have become available for the measurement of the concentration level of C-14, i. e., gas sample counting with a proportional counter and liquid scintillation counting. Although the latter is very useful for the measurement of

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1) Scientific Committee on the Effect of Atomic Radiation, A/AC. 82/R. 105; K. Kigoshi, This Bulletin, 33, 1576 (1960).

the radioactivity of many liquid samples, it has the characteristic weakness that the counting efficiency is strongly influence by the quenching of the sample. Unfortunately, many organic compounds act as quenching agents. Therefore, the solvent system which is used for this method can only measure the radioactivity of a limited number of organic compounds[2].

In the present work, the concentration of C-14 in thymol was measured by the liquid scintillation counting method; its annual variation was observed from 1954 to 1961.

Althouth thymol and other phenolic compounds could not be measured directly by this method because of the strong quenching effect arising from their phenolic nature, it was found that the quenching effect of phenols could be masked by the methylation of their phenolic hydroxyl group. Moreover, the methylation of thymol was carried out with a good yield.

Experimental

Sample Materials.—Thymol was obtained by the usual method[3] from the essential oil of *Orthodon japonicum* Benth (Labiatae). The plant, cultivated every year from 1954 to 1961, was harvested in October in the Experimental Field of Chiba University, located in Chiba city (35°35′N, 140°08′E). The methylation of the thymol was carried out according to the method used in the preparation of anisol[4]. Table I shows the yields of thymol from the essential oil and the yields arising from the methylation of the thymol thus obtained.

TABLE I. THE YIELDS OF THYMOL FROM ESSENTIAL OIL AND OF METHYLATION OF THE THYMOL THUS OBTAINED

Year	Oil	Thymol		Thymol methyl ether	
	g.	g.	(%)	g.	(%)
Pre-war[a]	84	41.4	(49.2)	24.0	(58.0)
1954	100	53.2	(53.2)	27.7	(75.6)
1955	—	—	—	—	—
1956	100	44.3	(44.3)	25.0	(71.4)
1957	90	49.2	(54.7)	25.2	(78.2)
1958	100	52.4	(52.4)	27.3	(81.4)
1959	100	46	(46.0)	28.9	(82.5)
1960	100	52	(52.0)	26.5	(75.7)
1961	150	80	(53.3)	30.3	(37.9)[b]

a) This was probably harvested during 1930—1940.

b) Raw product was used for methylation.

Preparation of Counting Samples. — 2,5-Diphenyloxazol (PPO) and 1,4-bis-(5-phenyl-2-oxazolyl)-benzene (POPOP) were dissolved in the thymol methyl ether obtained in concentrations of 0.4 and 0.01% respectively. Eighteen grams of these solutions were then put in low potassium glass vials (obtained from the Wheaton Glass Co., N. J., U. S. A).

Preparation of "Dead Carbon" Standard Samples. — *p*-Cymene and thymol methyl ether, consisting of the so-called "dead carbons" from petroleum products, were synthesized as standard samples in order to estimate the background counting rate. *p*-Cymene was prepared by the Friedel-Crafts reaction of toluene and isopropyl alcohol. Thymol synthesized from *m*-cresol and isopropyl chloride was obtained from the Koso Chemical Co., Tokyo, Japan, while its methyl ether was prepared in this laboratory.

Counting Procedure.—The measurement of radioactivity was carried out using a scintillation spectrometer Tri-Carb Model 314 A (Packard Instrument Co. Inc., Ill., U. S. A.) which incorporated a coincidence circuit and a two-channel pulse height analyzer. Each sample was counted 4 times for every 100 min. at a balance point operation with a 10~100 V. window setting. The counting efficiency of C-14 was calibrated by an internal standard of 5 mg. of benzoic acid (1-C-14). The specific activity is 5080 d. p. m. per mg. (Standard Source Compound, Packard).

Results and Discussion

Hayes et al.[5] recently reported the measurement of C-14 in *p*-cymene obtained from lemongrass oil with the aid of liquid scintillation counting, thereby proving that *p*-cymene could be used as an excellent liquid scintillation solvent. It is possible to synthesize *p*-cymene from thymol by elimination of its phenolic group[6], but the chemical yield of this dehydroxylation is poor (Chart 1).

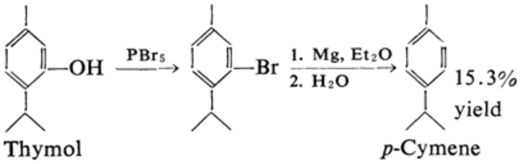

Chart 1. Synthesis of *p*-cymene from thymol.

On the other hand, the methylation of thymol is possible, giving a 70~80% yield (Table I); the strong quenching effect of thymol is almost masked by the methylation (Fig. 1). The counting efficiency of thymol methyl ether was found to be about 86% of that of toluene when it was used as a scintillation solvent.

The counting rates of samples prepared from natural products and from a "dead carbon"

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[2] F. N. Hayes, B. S. Rogers and P. C. Sanders, *Nucleonics*, **13**, 46 (1955); Y. Kasida, M. Yamazaki and T. Iwakura, *Radioisotopes*, **10**, 27 (1961).

[3] T. Kariyone and K. Atsumi, *Yakugaku Zasshi*, **40**, 707 (1920).

[4] L. Gattermann, "Die Praxis des Organischen Chemikers", 37 Aufl., Walter de Gruyter Berlin (1956), p. 212.

[5] F. N. Hayes, E. Hansburry, V. N. Kerr and D. L. Williams, *Z. für Physik*, **158**, 374 (1960).

[6] C. G. Le Fèvre, R. J. W. Le Fèvre and K. W. Robertson, *J. Chem. Soc.*, **1935**, 481.

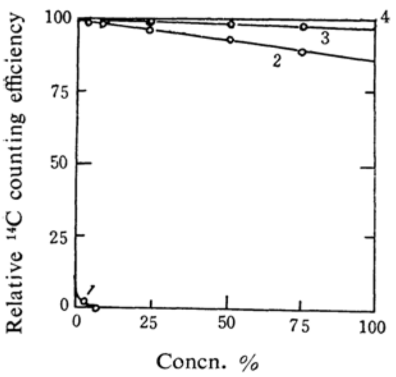

Fig. 1. Quenching curves of thymol and its related compounds.
1 Thymol 2 Thymol methyl ether
3 *p*-Cymene 4 Toluene

TABLE II. ANNUAL VARIATION OF C-14 CONCENTRATION IN THYMOL

Year	n_s	d. p. m./g. of carbon
Pre-war	123.47	13.62±0.14
1954	126.90	14.05±0.16
1955	—	—
1956	120.85	13.28±0.17
1957	140.85	15.84±0.17
1958	135.67	15.16±0.17
1959	148.89	16.82±0.17
1960	151.60	17.21±0.17
1961	154.29	17.49±0.17

$$\text{d. p. m./g. carbon} = \frac{n_s - n_b \pm 2sd}{C_g \times E}$$

$$sd = \sqrt{\frac{n_s + n_b}{t}}$$

n_s=Counts of samples, c. p. m.
n_b=Counts of natural background, 16.96 c. p. m.
$t = t_s = t_b$=Counting time, 400 min.
C_g=Carbon weight in thymol, 18 g. 13.16 g.
E=Counting efficiency, 59.4%

standard were 120∼150 c. p. m. and 17 c. p. m. respectively.

The data of measurement are listed in Table II. All the values of radioactivity are indicated in d. p. m./g. of carbon.

As is shown in this table, it was observed that the concentration of C-14 in thymol increased 18.4% from 1954 to 1961. This values was rather lower than the 26.8% which was reported by Hayes for the northern hemisphere⁵⁾. This difference suggests that the dilution effect by the "dead carbon" from fossil fuels probably contributes somewhat to the depression of the C-14 concentration. In fact, steel-manufacturing and other heavy industrial plants have gone into operation in the suburbs of Chiba city, and the consumption of coal in one of the big steel factories last year was about eight times that in 1953. Therefore, a considerable amount of "dead carbon" has been added to the atmosphere by the combustion of coal or oil; the C-14 concentration level might have been thereby reduced.

The pre-war sample shown in Table II was supplied from the Kasukabe Experimental Field of the National Institute of Hygienic Sciences, in Saitama prefecture (35°59′N, 139°45′E). This was harvested during the 1930—1940 period, but no definite harvesting data are known. The sample is used in the data as a supplemental reference to the level before the beginning of World War II.

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