Differentiation of Synthetic and Natural Caffeine

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C^{14} radioactivity of CO_2 derived from the combustion of caffeine has been used to differentiate synthetic from natural caffeine.

THE WIDE and varied use of caffeine in a large number of various products has placed such a demand for it against a shrinking supply from natural sources such as coffee, tea, and maté that for reasons of economy, manufacturers sometimes rely on synthetic processes to supply the demand. Because of policy reasons, it may be that a user wishes to use only the natural product. Until recently, there was no known method of analysis which would enable quality control chemists to differentiate synthetic from natural, vegetable caffeine. In December 1955, the author proposed the use of C^{14} radiocarbon analysis as a tool for making this differentiation. Knowledge of some of the steps in the commercial synthesis of caffeine indicated that the materials used would probably be derived, in part if not entirely, from petroleum or coal products, where the radioactivity of C^{14} had for all practical purposes become extinct. The half life of C^{14} is about 5500 years.

Radioactive C^{14} is produced by neutron bombardment of nitrogen in the upper atmosphere according to the equation

$$N^{14} + n = C^{14} + H^1$$

Neutrons are derived from cosmic rays originating in outer space. The C^{14} thus produced decays according to the reaction in which C^{14} loses a β^-

$$C^{14} \rightarrow N^{14} + \beta^-$$

particle and is changed to N^{14}. The discovery by Willard F. Libby of this phenomenon of radiocarbon formation and its subsequent distribution in our world is described in detail (4) and is a subject too extensive to treat more than briefly here. From his and other subsequent works it can be seen that the carbon reservoir of our world may be grouped into two categories. One is the living reservoir of animal, marine, and plant life and its environment, sometimes referred to as the biosphere and characterized by the processes of photosynthesis of carbon dioxide into organic matter by plant life and the metabolism of these products by animal

and marine life. The second category is the lifeless reservoir of coal, petroleum, and limestone which were supposedly made from organic matter in which life had ceased (metabolism had terminated) hundreds of thousands of years ago.

In the biosphere, the atmospheric CO_2 is used by plant life for photosynthesis of such compounds as cellulose, sugars, fats, and nitrogen-carbon vegetable compounds from which caffeine is derived. Recent evidence indicates that some of the carbon dioxide comes from soil. Atmospheric CO_2 consists of nonradioactive $C^{12}O_2$ (99%) and $C^{13}O_2$ (1%), and radioactive $C^{14}O_2$ in a concentration of the order of 10^{-12} relative to the total CO_2. The C^{14} which is formed continuously in the air by cosmic ray–triggered neutron bombardment of N^{14} is converted into $C^{14}O_2$ within a few hours and becomes mixed within the atmosphere by weather conditions.

All animal, marine, and plant life contains a consistent quantitative trace of radiocarbon C^{14} in their tissues. This concentration is approximately 1.3×10^{-12} relative to total carbon in tissues. There is no evidence of preferential absorption except in sea shells, which contain

about 3% more C^{14} than the life inside. This consistent trace has been assigned the title of 100% "modern carbon" in Table I. Inasmuch as the radioactivity of the modern carbon expires in an exponential manner, at the rate of half its intensity each 5500 years, the analysis of material from recently harvested crops should assay 100% modern carbon. Similar material synthesized from the second category sources would be without any modern carbon.

The production of C^{14} in the upper atmosphere is a process which is assumed to have been going on at a constant rate for many thousands of years, and as a result of this and the decay process which has transpired in category two, it is assumed that the two processes are in equilibrium. Recently, however, the C^{14}/C^{12} ratio of carbon has been lowered because of combustion of large quantities of C^{14}-free coal and petroleum during the past few decades. This decrease is estimated at about 2%. The many nuclear explosions on the other hand have operated so as to increase this ratio and will continue to do so for the next few years. This increase has been estimated to be 10 to 15%.

Table I. Radiocarbon Counts of CO_2 from Natural and Synthetic Caffeine

Sample No.	Place of Manufacture	Modern Carbon,[a] %	Remarks
133	Germany, mfg. A	0.0	Submitted as synthetic
295	Argentina	100.1	Submitted as natural from maté
304	Holland	90.1	Submitted as methylated natural theobromine
344	India	102.2	Submitted as natural from tea
419	Japan	104.6	Submitted as natural from tea
442	U.S.A., mfg. A	13.7	Submitted as synthetic
446	U.S.A., mfg. B	13.3	Submitted as synthetic
447	Germany, mfg. B	0.0	Submitted as synthetic
449	England	21.2	Submitted as synthetic
452	France	29.7	Submitted as mixture of synthetic and natural
489	Germany	104.8	Submitted as natural from coffee
501	U.S.A., mfg. A, Japan	72.8	Submitted as mixture made in Atlanta laboratory of 60% 419 and 40% 442 w/w

[a] Modern carbon, 100%, is normal proportion expected in recently harvested plants.

Conditions: Counter volume of 2 liters, $T = 65°$ F., $P = 1$ atm.; counting time ranged from 19 to 20 hours; background count ranged from 15 to 17 c.p.m.; probable error calculated to be $\pm 2.5\%$.

Some of the chemicals used in the manufacture of synthetic caffeine are urea, dimethylurea, and chloroacetic acid. For reasons of economy of manufacture, these chemicals are generally manufactured from petroleum and coal and their use from these sources would be the basis of the differentiation between synthetic and natural caffeine by the method used here. The application of C^{14} analyses to this problem was successfully done (results unpublished) in December 1956 in a series of experiments at the University of Michigan by Oliver Weinkauff of the Monsanto Chemical Co. and H. R. Crane of the University of Michigan. Subsequently, radiocarbon counting was done in other laboratories and recently known synthetic samples of caffeine from producers in America and Europe were analyzed by this method (1, 2) based on oxidation of the sample to carbon dioxide, purification of carbon dioxide, and subsequent radiocarbon counting of carbon dioxide with a proportional counter (3). Results of analyses are listed in Table I.

Discussion

Caffeine sold in commerce has been successfully analyzed by radiocarbon analyses for identification as to its synthetic or natural origin. The two samples from German synthetic producers indicate that both sources used carbon which is radioactively extinct. In the United States, the two sources used one modern carbon atom in the synthetic process. In England, the producer would appear to have used two modern carbon atoms.

A knowledge of industrial organic chemistry and synthetic organic methods is necessary in judging results of a C^{14} analysis for caffeine differentiation. An example of the need of knowledge is the use of dimethyl sulfate as a methylating agent in making caffeine from natural theobromine. In this step, one carbon atom is added. Methanol used for manufacture of dimethyl sulfate was formerly made by the destructive distillation of wood, but now it is usually manufactured by oxidation of petroleum hydrocarbons, or from hydrogen and carbon monoxide and carbon dioxide, which are also petroleum products. Wood-derived methanol would contribute modern carbon radioactivity in a manner similar to that of natural carbon in the methyl groups acquired from the atmosphere in nature's synthesis of vegetative matter. On the other hand, petroleum-produced carbon dioxide would not possess any radiocarbon and the methylating agent made from it would contribute a dead carbon to the caffeine molecule. Such is the case with sample 304 in the table, where there is 90.1% modern carbon.

Widespread use of basic chemicals derived from petroleum and coal in the manufacture of synthetic caffeine makes possible differentiation between synthetic and natural caffeine by this method. Application of this method obviously is not restricted to caffeine, but to any natural synthetic pair in which the synthetic one is made from dead carbon.

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COCOA POLYPHENOLS

Changes in Cocoa Tannins during Processing

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Three polyphenols of chocolate were separated by chemical fractionation and paper chromatography after different steps in the manufacturing process. These polyphenols were characterized by color tests and by ultraviolet and infrared spectra. One was identified as (−)-epicatechin; the other two are apparently similar compounds. Roasting diminished the concentration of (−)-epicatechin, and alkalizing or conching caused stereochemical changes in its structure. The other two compounds also underwent stereochemical changes during roasting; no further change occurred with alkalization, but conching reversed the change caused by roasting.

THE MANUFACTURE of both cocoa and chocolate products from cocoa beans is similar for the first few steps in processing. The fresh beans are fermented, roasted, and then ground to form chocolate liquor. In making cocoa by the Dutch process this liquor is treated with alkali and pressed to remove fat. The pressed cake is then pulverized. There are several processes for making chocolate. In the one which we have followed flavorings are added to the roasted beans, which are then refined and conched. A full description of these processes is given by Chatt (1). The detailed chemical changes which occur during such processes are generally unknown.

The polyphenols of fresh and fermented beans have been studied extensively by Forsyth and coworkers (2, 3, 4). In the fresh bean they have identified two anthocyanins, three leucocyanidins, (−)-epicatechin, (+)-catechin, (+)-gallocatechin, and (−)-epigallocatechin. During fermentation the anthocyanins were apparently largely converted to complex leucoanthocyanins. Schubiger et al. (9) reported that in addition to the com-