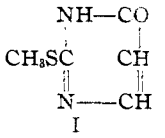
acteristic behavior toward chlorine gas in water and alcohol solutions.



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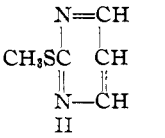

II

2. Pyrimidines of type I are desulfurized completely with formation of 2,4-diketohexahydropyrimidine compounds.

3. Pyrimidines of type II are oxidized to the corresponding 2-alkyl sulfonyl pyrimidine compounds. The double bond in positions 5 and 6 of the pyrimidine cycle is not altered.

NEW HAVEN, CONN RECEIVED APRIL 4, 1938

[CONTRIBUTION FROM THE FLEISCHMANN LABORATORIES, STANDARD BRANDS INCORPORATED]

The Volatile Constituents of Roasted Coffee

BY WILLIAM R. JOHNSTON AND CHARLES N. FREY

Our interest in the staling of roasted coffee led us to attempt to isolate and identify some of the aroma and flavor constituents. It is evident that any effort to prevent or retard the staling of roasted coffee can be undertaken with better prospects of success if the identity and nature of the substances responsible for its flavor and aroma can be more completely ascertained.

When we started our investigation the best information on the subject was contained in the patents issued to Staudinger and Reichstein.[1] They outlined a method of isolating coffee volatiles by vacuum distillation and listed a number of compounds supposedly present in roasted coffee. Some of the compounds reported were: hydrogen sulfide, methyl mercaptan, furfuryl mercaptan, dimethyl sulfide, acetaldehyde, furfuraldehyde, diacetyl, acetyl-propionyl, furfuryl alcohol, acetic acid, guaiacol, vinyl guaiacol, pyridine, pyrazine and N-methylpyrrole. However, in British Patent 260,960, the statement is made that the compounds reported were not actually found in coffee but were split products obtained by analysis—the real products being unstable and difficult to isolate. This statement is somewhat confusing since some of the substances mentioned have been reported previously by earlier investigators as being present in coffee. For example, pyridine, furfuraldehyde, furfuryl alcohol and acetic acid actually have been isolated from roasted coffee. The older literature on this subject has been surveyed critically by Prescott and his co-workers.[2] With this contradiction in mind, we decided to use the work of Staudinger and Reichstein as a guide and attempt to isolate the volatile coffee components as nearly unchanged as possible. Their procedure includes distillation at 2–5 mm. pressure at 100–110° and the use of steam or liquid water to assist in removal of volatile substances from roasted coffee. We believed that this treatment was not ideal because previous work on staling had convinced us that very small amounts of oxygen were sufficient to cause appreciable deterioration of the coffee and in addition that moisture had a distinct accelerating effect on this deterioration. Consequently, we decided to utilize a high vacuum apparatus and to avoid the use of water during the distillation. By distilling at low pressures and working in the practical absence of oxygen we have been able to isolate as actual constituents of coffee several of the substances reported by Staudinger and Reichstein. We also have detected methylacetylcarbinol as a new constituent of roasted coffee.

Shortly after we had completed our investigation, two excellent papers by Prescott and his associates[2,3] supplied valuable new information on the constituents of roasted coffee. Prescott and his collaborators relied on solvent extraction of coffee brew and dry roasted coffee to isolate several new substances which were identified in the usual manner. The following substances were reported: furfuryl alcohol, furfuraldehyde, acetic acid, formic acid, diacetyl, diethyl ketone, kahweol, vanillone, *p*-vinylguaiacol, guaiacol, *n*-heptacosane, *p*-vinylcatechol, sylvestrene, eugenol, and a hydrocarbon melting at 116–117°. Of these substances, formic acid, diethyl ketone, vanillone, *n*-heptacosane, *p*-vinylcatechol, sylves-

(1) Staudinger and Reichstein, British Patents 246,454 and 260,960.

(2) Prescott, Emerson and Peakes, *Food Research*, **2**, 1–20 (1937).

(3) Prescott, Emerson, Woodward and Heggie, *ibid.*, **2**, 165–173 (1937).

trene, eugenol, and the hydrocarbon (m. p. 116–117°) have not been mentioned specifically by previous workers.

Judging from this work and from our own it is likely that the substances reported by Staudinger and Reichstein were not split products but actual coffee constituents.

Although not directly concerned with coffee, the paper by Reichstein and Beitter[4] on the aroma constituents of roasted chicory is well worth the study of anyone interested in the chemistry of coffee. The methods of isolation and identification described by Reichstein and Beitter are very probably quite similar to those used previously by Staudinger and Reichstein.

Apparatus and Procedure

Dry Distillation of Roasted Coffee (Santos).—The high vacuum distillation equipment included a heavy copper flask of about 15-liters capacity which was suitable for holding a charge of 15 kg. of finely ground coffee; a Pyrex glass manifold carrying 3 receivers of 200-cc. and 3 of 100-cc. capacity, a trap for mercury vapor, a mercury vapor pump, and a Cenco Hy-vac pump as a backing pump.

After charging the copper distillation flask with coffee it was fitted with a rubber stopper carrying a glass tube which was sealed to the receiver manifold. The joints between the rubber stopper, copper and glass were sealed vacuum tight with hard deKhotinsky cement.

The larger receivers were cooled with solid carbon dioxide and the smaller ones with liquid air. Each was fitted with a ground joint which permitted the removal of the lower portion holding the condensate.

In carrying out the distillation, after all leaks had been eliminated, the pumps were operated for several hours to remove all of the oxygen in the system. During this time the distillation flask was at room temperature but even at this temperature appreciable amounts of carbon dioxide, water vapor, and volatile aroma and flavor constituents condensed in the receivers. Only traces of substances condensed in the final liquid air trap and only small amounts of gas, presumably carbon monoxide, passed this receiver, since the pressure at that point was usually about 0.005 mm. The pressure in the copper flask could not be measured at any time but since the bore of the manifold and receiver tubes was of the order of 1 cm. at all points, it is probable that no considerable drop in pressure existed between the distillation flask and the last receiver.

When oxygen had been eliminated completely from the system, the distillation flask was heated gradually to 100° on the water-bath and maintained at that temperature throughout the distillation. Condensate accumulated rapidly after the coffee was heated, most of the water condensing in the receivers cooled with solid carbon dioxide, while carbon dioxide, traces of water, and a small amount of a pale yellow liquid having a strong coffee aroma condensed in the liquid air receivers.

When it became necessary to remove a receiver, oxidation of the condensate was avoided by filling the manifold with pure nitrogen. After removal from the apparatus the receiver was sealed and stored in a packing of solid carbon dioxide until analysis of the condensate was undertaken. The distillation was continued until practically no more liquid condensed in the solid carbon dioxide receivers. This usually required about forty hours. After one charge of coffee had been distilled, the copper flask was removed, a fresh charge introduced, and the distillation continued, following the procedure just outlined. In a typical experiment in which 100 lb. (45 kg.) of coffee was distilled, about 850 cc. of condensate in the solid carbon dioxide receivers and approximately 50 cc. of condensate in the liquid air receivers were obtained. The amount of liquid air condensate (50 cc.) is exclusive of the condensed carbon dioxide which was not retained.

Examination of Condensates.—The condensate from the receivers cooled with liquid air was allowed to volatilize at atmospheric pressure, the effluent gas, chiefly carbon dioxide, being passed through receivers cooled with solid carbon dioxide. The gas finally escaping smelled strongly of hydrogen sulfide and of mercaptans or organic sulfides. Testing with lead acetate identified the hydrogen sulfide beyond question. The residual condensate (50 cc.) was a dilute aqueous solution supporting a thin layer of a pale yellow liquid. This mixture was distilled at atmospheric pressure on the water-bath at 75° in a slow stream of nitrogen until about 2 cc. of a pale yellow liquid condensed in a receiver cooled with solid carbon dioxide. The residual aqueous solution was combined with the primary condensate obtained in the three receivers cooled with solid carbon dioxide.

The pale yellow liquid was redistilled on a water-bath in an atmosphere of pure nitrogen, using receivers cooled with solid carbon dioxide. Three fractions were taken distilling at 25–35, 35–50 and 50–85°, only a trace of brownish residue remaining in the distilling flask. The first fraction (0.4 cc.) was mostly soluble in water and had an odor of acetaldehyde. This substance was identified by formation of its 2,4-dinitrophenylhydrazone, m. p. 162–164°. A mixed melting point with an authentic sample confirmed the identification. Judging by the amounts of hydrazone formed, acetaldehyde was the chief ingredient of this fraction. A trace of furan seemed to be present since a pine splinter gave a green reaction and a brown precipitate was obtained on treatment with concentrated hydrochloric acid.

The second fraction (0.8 cc.) also contained acetaldehyde and, in addition, a mixture of perhaps several aldehydes and ketones, for a mixture of 2,4-dinitrophenylhydrazones was obtained. An attempted separation was not successful because of the small amount of material available.

The third fraction (0.4 cc.) was found to contain a small amount of diacetyl which was isolated as a *bis*-semicarbazone, m. p. 281–282°. A mixed melting point of 281–282° confirmed the identification. The third fraction had a good coffee-like aroma and also a mercaptan-like odor. Sulfur was detected but mercaptans or sulfides could not be isolated or identified. A ketone was isolated in the form of a red 2,4-dinitrophenylhydrazone, m. p. 258–259°, but its identification was not completed, again because of the minute amounts of material available.

(4) Reichstein and Beitter, *Ber.*, **63B**, 816–826 (1930).

The primary condensate from the receivers cooled with solid carbon dioxide contained droplets of oil. Immediately on exposure to air the oil droplets turned reddish-brown and the solution gradually darkened and deposited a brownish resin or tar. This also took place when the condensate was heated to 100–110° in pure nitrogen. The oxidized oil droplets had an odor very similar to that of very stale coffee.

From a portion of the condensate diacetyl was precipitated as its characteristic osazone, m. p. 243–245°, the identification being confirmed by a mixed melting point with the osazone obtained from an authentic sample of diacetyl.

The bulk of the condensate was separated from the oil droplets and acids and then roughly fractionated. It was first distilled at about 50–60 mm. pressure in a slow stream of pure nitrogen to remove the water. After practically all the water had distilled over, the distillation was stopped and the oily residue sealed in nitrogen to be saved for further work. The aqueous distillate was saturated with potassium carbonate and extracted with pure ethyl ether. The ether extract was concentrated to yield a viscous brown oil (3–4 cc.) which was distilled at 20–30 mm. in a stream of nitrogen. Two fractions were taken: A (1 cc.) distilling at 20–35°; and B (1 cc.) 35–65°; and a residue of about 2 cc. was set aside. Fraction A was found to be mainly a mixture of aldehydes and ketones and was not investigated further. Fraction B had an odor of pyridine and yielded a picrate melting at 167–168°. A mixed melting point with an authentic sample of pyridine picrate confirmed the identification.

Fraction B was fractionated at atmospheric pressure in pure nitrogen to yield a main fraction of 0.5 cc. boiling at 145–165°. This distillate gave a phenylhydrazone melting at 97° which proved to be the phenylhydrazone of furfuraldehyde. A mixed melting point clinched the identification. The aniline acetate test was also positive.

A few drops of a higher boiling liquid were also obtained from fraction B which seemed to be furfuryl alcohol but the identification was not confirmed by preparation of a derivative.

The distillate from fraction B coming over at 145–165° also yielded a phenylhydrazone melting at 237–240° which on purification melted at 244–245°. A mixed melting point with diacetyl osazone was 244–245°, so it was evident that either diacetyl or methylacetylcarbinol must have been present in fraction B. But diacetyl is excluded by its boiling point so the compound was identified as methylacetylcarbinol which boils at 148° and yields diacetyl on oxidation. Its isolation may be ascribed to the care with which oxygen was excluded during manipulations. Its oxidation to diacetyl occurred during the preparation of the osazone in the presence of air.

Tests on the higher boiling fractions obtained from the aqueous condensate and on the small amount of water insoluble oils, indicated that phenols, nitrogenous compounds other than pyridine and high boiling aldehydes and ketones were present. The phenolic fractions had a coffee-like odor and were very sensitive to heat and oxygen.

Discussion

In the main, our work has been an extension, a general confirmation, and an elucidation of the patent disclosures of Staudinger and Reichstein. We have found high vacuum distillation to be very suitable for isolating the volatile coffee constituents and have isolated several coffee constituents which have been reported by Staudinger and Reichstein to be split products and not actual constituents. As mentioned previously, the indications are that most if not all of the substances listed by Staudinger and Reichstein are actually present in coffee.

The identification of diacetyl, furfuraldehyde, pyridine, and furfuryl alcohol further confirms previously published reports. The detection of acetaldehyde, furan and hydrogen sulfide confirms the work of Staudinger and Reichstein, but the isolation of methylacetylcarbinol is new. Separation of fractions containing phenols, nitrogenous compounds other than pyridine, and mixtures of aldehydes and ketones again confirms several previous investigations, but the detection of sulfur in a volatile fraction is the first confirmation in this respect of Staudinger and Reichstein's work. We are inclined to agree with their belief that the compounds containing sulfur are of major importance with respect to the distinctive character of coffee aroma. The fraction containing sulfur had a very intense odor which seemed to embody the unusual aroma and taste sensations imparted by roasted coffee.

The work of Prescott and his associates, mainly on the less volatile constituents of coffee, also confirms certain aspects of the researches of Staudinger and Reichstein and is of great importance in understanding the changes which take place on staling. We are in complete agreement with Prescott with respect to the probable changes occurring during the staling process Staling probably involves volatilization, oxidation, hydrolysis, and polymerization of the various flavor and aroma constituents, and has little if anything to do with the fat of coffee.

At the present time we have an imposing list of coffee aroma and flavor constituents which should be of great assistance in studying the staling of coffee and various other problems of the coffee industry.

Summary

The utilization of high vacuum distillation to isolate the volatile aroma and flavor components of roasted coffee is described. The identification

of diacetyl, methylacetylcarbinol, furan, furfuraldehyde, furfuryl alcohol, acetaldehyde, pyridine, and hydrogen sulfide is reported. The contributions of Staudinger and Reichstein and of Prescott and his associates are discussed and the relation of the volatile constituents to the staling of coffee is considered briefly. Coffee staling is probably concerned with changes in the volatile aroma and flavor substances and does not involve fat rancidity.

810 GRAND CONCOURSE
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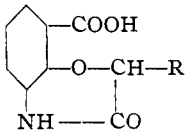
[CONTRIBUTION FROM THE MELLON INSTITUTE OF INDUSTRIAL RESEARCH AND E. R. SQUIBB AND SONS]

The Preparation of 2-Methyl-8-carboxy-3-keto-3,4-dihydro-1,4-benzoxazine

BY HAROLD W. COLES[1] AND WALTER G. CHRISTIANSEN[2]

In the search for new analgesics without the chemical structures said to cause agranulocytosis[3] we have been interested in the possibilities of the benzoxazine ring system, and particularly in the 8 - carboxy - 3 - keto - 3,4 - dihydro - 1,4 - benzoxazines (I) since it is seen that these compounds



I

may be considered as derivatives of salicylic acid, and they also contain the skeleton of acetanilide. The carboxyl group not only acts in a detoxifying capacity, but also permits the preparation of a soluble sodium salt.

A number of carboxylated benzoxazines have been reported in the literature[4] but, so far as we know, were not tested pharmacologically. Therefore, 2-methyl-8-carboxy-3-keto-3,4-dihydro-1,4-benzoxazine (I, R = CH_3) was synthesized and, being physiologically inactive, no further members of this series were prepared.

The authors wish to record their appreciation for the interest and advice of Dr. George D. Beal, Assistant Director of Mellon Institute, during the progress of this work.

Experimental Part

3-(α-Bromopropionylamino)-salicylic Acid.—The procedure, here described, is adapted from directions given by Sanna[5] for the non-carboxylated aminophenols. One mole equivalent (4.25 g.) of 3-aminosalicylic acid and slightly more than one mole equivalent (6.25 g.) of α-bromopropionyl bromide was added to 50 g. of dry benzene. The aminosalicylic acid remained suspended in the benzene since it is quite insoluble. The Erlenmeyer flask contents were refluxed on a water-bath for twelve to fourteen hours with the evolution of acid fumes. The flask contents were chilled and the grayish crystalline material was removed to a Büchner funnel and washed repeatedly with cold benzene. The crystals, after drying in the air, weighed 8 g. representing a practically theoretical yield. It was recrystallized from a minimum of boiling 50% alcohol, washing repeatedly on the filter with distilled water. It was dried in an 80° oven. The white solid has an irritating odor, and in a melting point tube it softened quite sharply at 178°, but the column did not break until 188° (U. S. P. corr.).

Anal. (Kjeldahl). Calcd. for $C_{10}H_{10}O_4BrN$: N, 4.86. Found: N, 4.64.

The crystals are easily soluble in acetone, alcohol and alkalies, but sparingly soluble in water and benzene.

2 - Methyl - 8 - carboxy - 3 - keto - 3,4 - dihydro - 1,4-benzoxazine (I, R = CH_3).—3-(α-Bromopropionylamino)-salicylic acid (3.3 g.) was dissolved in 25 cc. of 10% sodium hydroxide solution and warmed on the water-bath at 60° for one hour. The color darkened considerably. The solution was cooled, filtered and dilute (1:1) hydrochloric acid added until the dark solution was acid to congo red. A dark precipitate came out on standing, and was washed repeatedly with distilled water. Recrystallized from a minimum of boiling absolute alcohol, the product was secured as colorless crystals (yield 60%). The substance is sparingly soluble in the usual solvents. It is readily soluble in alkalies; m. p. 285° (U. S. P. corr.).

Anal. Calcd. for $C_{10}H_9O_4N$: N, 6.61. Found: N, 6.76.

Pharmacological Tests.[6]—6-Carboxy-3-keto-3,4-dihydro-1,4-benzoxazine[4a] and 2-methyl-8-carboxy-3-keto-3,4-dihydro-1,4-benzoxazine, administered intravenously to mice, rats and rabbits, exhibited no antipyretic or hypnotic action in doses up to 2000 mg. per kg. The first compound shows some toxic effects above 500 mg. per kg., while the second is non-toxic at least up to a dosage of 1500 mg. per kg. The compounds were not tested as to possible local anesthetic properties. The effect of the introduction of the carboxyl group into the benzene ring is very marked

(1) Senior Industrial Fellow, E. R. Squibb and Sons Industrial Fellowship, Mellon Institute.

(2) E. R. Squibb and Sons, Brooklyn, N. Y.

(3) Council on Pharmacy and Chemistry, A. M. A., *J. Am. Med. Assoc.*, **102**, 2183 (1934).

(4) (a) Christiansen, THIS JOURNAL, **47**, 1158 (1925); **48**, 460 (1926); (b) Einhorn and Oppenheimer, *Ann.*, **311**, 154 (1900).

(5) Sanna and Vacca, *Gazz. chim. ital.*, **62**, 555 (1932); Puxeddu and Sanna, *ibid.*, **61**, 158 (1931).

(6) The authors are greatly indebted to the Biological Laboratories, E. R. Squibb & Sons, New Brunswick, N. J., for these tests.