New Developments in Coffee Aroma Research

F. GAUTSCHI, M. WINTER, Y. FLAMENT, B. WILLHALM, AND M. STOLL

A large number of substances were isolated and identified from a concentrated coffee aroma obtained by molecular distillation of expelled coffee oil. Enrichment of small constituents by mild fractional distillation, liquid-liquid partition, and column chromatography prior to analysis by gas chromatography has led to the identification of constituents present in concentrations down to 0.5 p.p.m., with respect to the starting concentrate. Investigation by spectrometry combined with synthetic work was used as the main tool for identifications, assisted in some cases by IR and UV spectrometry. The identification of 22 constituents is described to illustrate the technique used. Twenty of these compounds have not yet been found in coffee; possible ways of their formation during the roasting process are mentioned briefly.

The chemical nature of coffee aroma has been a challenge to the chemist for a long time. Interest in this field has increased in recent years, thanks to the development of new analytical tools and in view of the growing importance of instant coffee which presents problems with respect to retention of the full flavor contained in freshly roasted and ground coffee. A study of recent literature shows that widespread use has been made of both gas chromatography and mass spectrometry for stimulating new work in this field. No attempt is made here to survey recent work on the subject; for pertinent references see Gianturco, Giammarino, and Friedel (8) as well as Merritt and Robertson (13).

This paper presents part of the authors' results obtained on the analysis of coffee flavor over a period of several years, special emphasis being given to the technique of identification. The summarized work will be published shortly in *Helv. Chim. Acta* (16).

The aroma of coffee originates from a large number of substances blended together, including substances of high and medium volatility.

The starting material chosen consisted of a coffee concentrate obtained by molecular distillation of expelled coffee oil; the same kind of material has been used by Gianturco and Giammarino (7). This concentrate is a yellow liquid which is highly unstable and darkens very rapidly; when stored in liquid nitrogen, it remains stable for several weeks. GC investigation of this material shows that about 50% is made up of very volatile compounds; major constituents are lower aliphatic aldehydes, acetic acid, and pyridine. This volatile part of the concentrate resembles headspace aroma of freshly ground coffee, as also was pointed out by Gianturco and Giammarino (7).

The analytical work carried out on the less volatile part is illustrated below by the identification of certain compounds or classes of compounds not yet known in the literature of coffee aroma.

Firmenich & Co., Geneva, Switzerland

Procedure

As has been demonstrated (8, 13), the difficulties encountered in the analysis of coffee aroma are due to its very complex nature. Some 200 constituents are present in widely different concentrations. Direct GC investigation of the more volatile ones is feasible, although repeated passages through columns of different polarity are necessary to get down to individual substances. Separation of the less volatile constituents proved to be more delicate. As GC techniques were not very well developed at the time work was started, other methods were combined successfully with them—mild fractional distillation was used to remove the most volatile part and the remainder was treated by different procedures in order to enrich particularly characteristic subfractions of the aroma. Final separation into pure constituents was accomplished by repeated GC. Attempts to separate the starting mixture chemically into acids, bases, phenols, and neutral substances were not very promising because of the presence of weak acids and bases of rather lipophilic nature coupled with the hydrophilicity of other constituents which made clear-cut separations impossible. Also, little success was met in trying direct derivative formation of carbonyls, acids, or phenols on the same mixture; contamination with artifacts occurred and minor constituents could not be detected. The methods finally adopted in combination with GC were column chromatography using supports of low activity, selective extraction of interesting substances with pentane, and countercurrent partition to separate fractions of different polarity (16). Programmed temperature and preparative GC became available during the work and were used extensively. Minor constituents representing as little as 0.5 p.p.m. of the starting coffee concentrate could be isolated only with the use of concentration steps.

Identification was based largely on spectroscopic methods. Mass spectrometry (MS) proved to be the most important help, combined in some cases with IR or UV spectrometry. Experience gained in MS

during the work permitted identification of several compounds still present in impure form after several separation steps. The instrument used was an Atlas CH-4 spectrometer, ion-source temperature, 250° C., inlet temperature 150° C., electron energy 70 e.v., ionizing current 16 or 35 μa. Synthetic work was necessary in all cases where reference substances were not available to verify MS interpretations.

Results and Discussion

The products described below have been chosen because of their interest in connection with the identification work involved, or because they are representatives of substances not well known at present among coffee constituents. Some comments are given concerning possible ways of formation from precursors existing in the green bean.

Terpene-Like Constituents. Figure 1 shows compounds identifiable in different subfractions. With the exception of linalool (II) which could be isolated in sufficient amount to obtain an IR spectrum, all of them were present in very small amounts. MS interpretation was facilitated by the typical odors exhibited and the available spectra of a number of such substances in our laboratory: Myrcene (I) was detected easily among a number of simple hydrocarbons; α-terpineol (III) occurred together with linalool (II) and was separated by repeated GC. The two oxides of linalool, IV and V, had been found in connection with work on essential oils (6) and the mass spectra of the products found in coffee were in agreement with the ones of the synthetic products; additional proof was given by GC retention times.

The tetrahydrofuran structures of IV and V might indicate that some of the numerous furans to be found in coffee originated from such precursors rather than being derived from carbohydrates. Compounds I to V probably are formed during roasting by thermal breakdown of higher terpenes which are known to occur among the lipids of the green bean, among which cafestol, kahweol, and squalene are described (11). This hypothesis is supported by the presence of hexahydropseudoionone (VI) and 6,10,14-trimethylpentadecane-2-one (VII). Having found a number of higher aliphatic ketones in the fraction containing VI, MS interpretation led to its formula confirmed by the preparation of a synthetic reference substance through hydrogenation of a sample of pseudoionone. Identification of VII was coincidental, as a sample of VII had been isolated in work connected with constituents of jasmine (5), allowing positive proof of structure with respect to the presence of this ketone in coffee.

Furan Derivatives. Figure 2 shows some furan compounds found in this work which represent interesting features with respect to identification. The presence of furan derivatives in coffee aroma is well known, the simplest being furan and 2-methylfuran (12). Higher furans occur as well—such as 2,2'-difurylmethane,
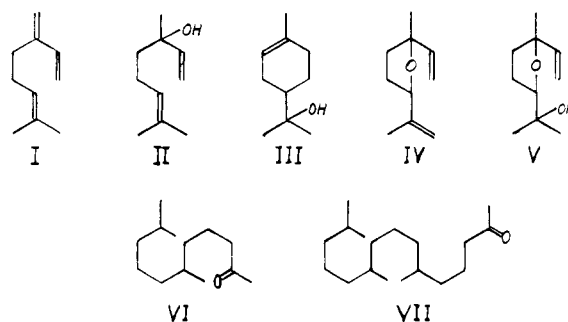

Figure 1. Terpene-like constituents

Myrcene (I), linalool (II), alpha-terpineol (III), "anhydrolinalool-oxide" (IV), "linalool-oxide" (V), hexahydropseudoionone (VI), 6,10,14-trimethylpentadecane-2-one (VII)
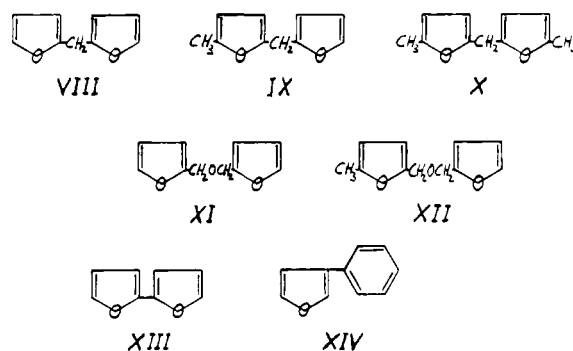

Figure 2. Furan derivatives

2,2'-Difurylmethane (VIII), 5-methyl-2-furfurylfuran (IX), di-[5-methylfuryl-(2)]-methane (X), difurfurylether (XI), 5-methyl-difurfurylether (XII), 2,2'-bifuryl (XIII), 3-phenylfuran (XIV)

mentione by Reichstein, Grüssner, and Zschokke (14), and proved to be a coffee constituent by Gianturco, Giammarino, and Friedel (8). This compound was found independently in this work, being a major peak in a nonpolar subfraction. An IR spectrum could be recorded in addition to the MS. Interpretation of these data (8), supported by Reichstein's citation, led to formula VIII. The product was synthesized (1, 3) and proved to be identical (IR, GC, MS) with the coffee constituent. It was quickly realized that homologs of VIII were present in the same subfraction. Another major peak examined by IR and MS showed a molecular weight of 162, and the striking similarity of its IR and MS with those of VIII immediately suggested the presence of a methyl-homolog (IX); this was confirmed by synthesis (Table I).

A third smaller constituent had a molecular weight of 176 according to MS. Its fragmentation pattern was related to the one of VIII and IX, indicating the presence of a dimethyl homolog of VIII. According to literature, such compounds are formed by internal condensation of furfuryl alcohol under acidic conditions, or by reaction of furan or 2-methylfuran with formaldehyde (1, 3); all of these compounds are

known to be present in coffee aroma. They derive from the carbohydrates of the green bean and may condense partly during roasting. Synthesis (1, 3) permitted the identification of VIII, as well as of IX and X—namely, 5-methyl-2-furfurylfuran and di-[5-methylfuryl-(2)]-methane. Self-condensation of furfuryl alcohol produces, in addition, difurfuryl ether (XI). This compound was isolated as a major component from several subfractions, and characterized by IR and MS (Table I). The MS is interpreted very easily; the furfuryl fragment at mass 81 is the predominant feature of this spectrum which, taken with the parent peak at mass 178, suggests structure XI convincingly. Again, compound XI was accompanied by a small amount of a methyl homolog, for which structure XII seemed most likely. Synthesis of this product was accomplished by side-chain bromination of 2,5-dimethylfuran, followed by reaction of the monobromide with the alcoholate of furfuryl alcohol. The synthetic ether showed the MS expected which

was identical with the one of the natural product.

Compound XIII represented another major constituent within the fraction containing VIII. Again, IR and MS information could be obtained. Its molecular weight was 134 according to MS, suggesting the global formulas of $C_9H_{10}O$, $C_8H_6O_2$, or $C_8H_{10}N_2$. The IR spectrum revealed a sharp band at 3.18 microns which is typical of furan C—H stretching absorption. A rather stable structure is indicated by the fact that the base peak is equal to the parent peak; no furfuryl fragment is visible. Consideration of these data led to 2,2'-bifuryl as a valid hypothesis. This compound was synthesized following Reichstein (14). As expected, 2,2'-bifuryl proved to be identical with the original coffee product. Figure 3 shows the MS, IR, and UV spectra of both natural and synthetic substances. A UV spectrum was recorded in this case since such a heterocyclic polyene exhibits fairly typical UV behavior.

There remains compound XIV in this group which

Table I. Mass Spectral Data of Coffee Furans—Natural vs. Synthetic Sample

| m/e	VIII Synth.	VIII Nat.	IX Synth.	IX Nat.	XI Synth.	XI Nat.	m/e	VIII Synth.	VIII Nat.	IX Synth.	IX Nat.	XI Synth.	XI Nat.
26			0.9	1.8			89	3.2	3.5	1.9	1.8		
27	13.4	13.8	13.4	16.4	9.7	11.3	90	2.6	2.5				
29	3.5	3.8	0.9	2.9	2.3	3.4	91	92.2	89.6	53.2	50.4		
38	5	5	1.6	2.1	1.3	1.6	92	12.2	12	5.1	5		
39	21	20.8	9.5	11.4	8.2	10	94	14.6	13.9	1.4	1.8		
40	2	2.5					95			8.3	8.6	3.3	4.2
41	1.8	2.2	3.2	6.1	4.9	6	96					1.3	1.8
43			38.5	37.5		1.3	97					8.7	9.5
45	1.2	1.9					98				5.7		
46	3.5	4.4					103			2.8	2.5		
50	4.1	4.1	3.7	4.3	1	1.3	105	1.8	2.2	9.5	9.3		
51	10.5	10.4	11.1	11.4	2.8	3.4	108			8.6	7.9		
52	4.7	4.4	6.7	9.3	2.3	2.9	110					3.8	4.2
53	14.6	14.5	13.9	17.5	19.7	21.3	119	16.9	17	23.3	22.5		
54					4.3	4.7	120	25.6	25.8	4.4	4.3		
55	4.7	5	2.5	3.2			121	2	2.2				
57				4.3			125						1.6
60	1.2	1.9					131	0.9	1.3				
62	1.8	2.2					133			7.6	7.5		
63	7.3	7.3	3.5	3.6			134			5.1	5		
64	1.5	1.9					147	20.1	20.5	24.1	22.8		
65	18.6	18.6	8.8	8.6			148	100	100	1.9	1.8		
66	4.1	4.1	3.5	3.9			149	9.9	9.8				
67	1.2	1.6	1.2	1.8			161			24.1	22.5		
68	5.8	6	0.9	1.1			162			100	100		
69					3.8	4.2	163			11.1	11		
74	2.6	2.8					178					6.7	6.8
77	1.5	1.9	4.6	5			179					0.7	0.7
78	1.2	1.6	1.4	1.4			182				2.9		
79	1.2	1.6	5.6	5.7									
80			2.3	3.9	1.8	2.1							
81	12.8	12.6	13	36.8	100	100							
82			2.3	3.9	46.8	47.8							
83					2.6	3.4							
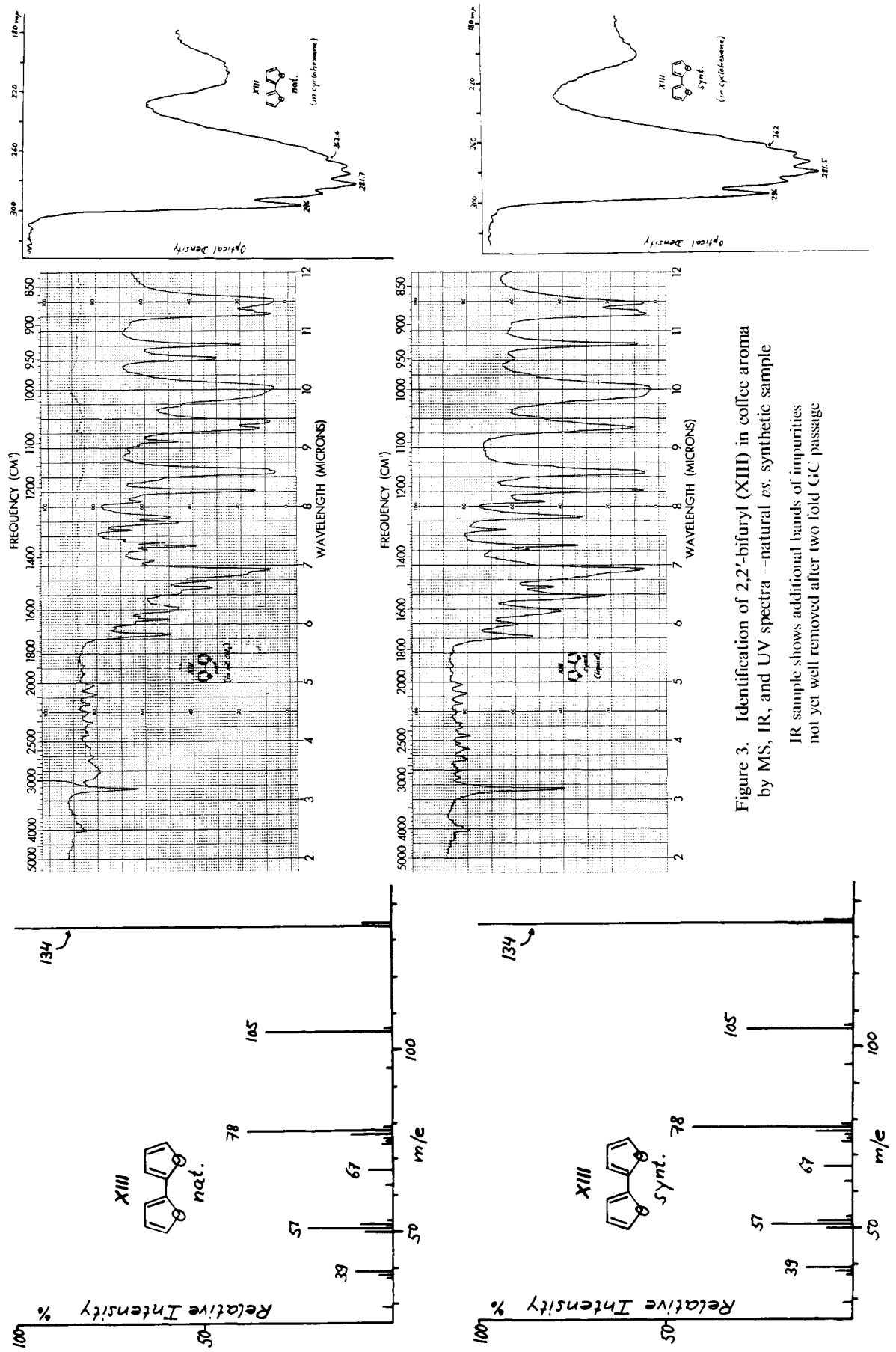
Figure 3. Identification of 2,2'-bifuryl (XIII) in coffee aroma by MS, IR, and UV spectra –natural vs. synthetic sample

IR sample shows additional bands of impurities not yet well removed after two fold GC passage
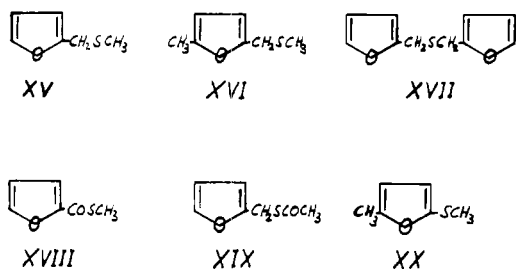
Figure 4. Furan-sulfur compounds

Furfurylmethyl sulfide (XV), 5-methylfurfuryl-2-methyl sulfide (XVI), difurfuryl sulfide (XVII), methylthiofuroate (XVIII), furfuryl thioacetate (XIX), 2-methylthio-5-methylfuran (XX)

Table II. Mass Spectral Data of 3-Phenylfuran (XIV), Natural and Synthetic, Compared with Dehydronaphthalene-1,4-endoxide (DNO)

m/e	XIV Synth.	XIV Nat.	DNO Synth.
38			1.8
39	5.3	4.9	8.7
50	2.5	2.5	4.7
51	3.5	3.5	5.3
57			3.5
57, 5	1.8	1.4	5
58			4.3
62	3.2	3.2	5.3
63	8.8	8.8	13.2
64	1.1	1.1	2.2
65	3.9	4.2	4.3
72	4.9	4.6	4.3
74	1.4	1.4	3.2
75	1.4	1.4	3
86	1.1	1.1	1.5
87	1.4	1.4	2
88	1.1	1.1	2
89	8.5	8.5	13.2
90			4
113	1.1	1.1	2
114	2.8	2.8	6.5
115	79	80	100
116	8.8	8.8	66
117			5.3
118			18
119			1.2
128			5.3
144	100	100	61.7
145	10.9	11	6.7

represents also an important peak in a nonpolar subfraction. The product, when trapped after GC, crystallized readily (m.p. 56–57° C.) and had an odor reminiscent of that of an aromatic hydrocarbon. Its IR and MS could be recorded; molecular weight according to MS is 144. A first hypothesis was based on a formula $C_{11}H_{12}$, and an ethylindene was considered as a possible structure. 1-Ethylindene is easily synthesized, starting from β-ethylhydrocinnamic acid, followed by cyclization to the corresponding indanone, and subsequent reduction and dehydration. A small amount of 1-ethylindene mixed with isomeric 3-ethylindene was prepared by this procedure. Its MS, however, showed clearly that no such structure was possible for the coffee product (unlike the MS of the natural product of Table II, both isomers have base peaks at m/e 129: loss of CH_3). Re-examination of the MS of the latter showed that a formula $C_{10}H_8O$ was more likely, considering the abundance of the $M + 1$ peak caused by ^{13}C isotope content (2). A literature search revealed dihydronaphthalene-1,4-endoxide melting at 56°–57° C. as another good hypothesis since this product could form during roasting by interaction of furan with an activated form of benzene. The synthesis of this oxide actually passes through a benzyne intermediate generated from o-fluorobromobenzene in the presence of methyllithium which undergoes a Diels-Alder condensation with furan (9). Following this work, the naphthalene derivative was prepared. Its MS is given in Table II, together with that of the natural product: despite some resemblance there is no doubt that the synthetic product does not correspond to XIV. Additional literature search yielded 3-phenylfuran which has been described only recently (17). It melts at 57–58° C. and its furan moiety explained very nicely bands in the IR spectrum of natural XIV which had been unclear before. 3-Phenylfuran was synthesized following (17) and proved to be identical with natural XIV (Table II).

Furan-Sulfur Compounds. Furfuryl mercaptan has attracted considerable attention for coffee flavor since its discovery by Reichstein and Staudinger (15). No other representative of furan sulfur compounds has been described in the literature until the recent work of

Gianturco and coworkers (8), who were able to demonstrate the presence of furfurylmethyl sulfide (XV) in coffee. Such compounds may be formed during the roasting of coffee by interaction of degradation products from proteins with carbohydrates. Analysis of certain nonpolar subfractions of the starting material used suggested the presence of several sulfury notes exhibiting strong odors; all of them occur in very small amounts only. It was found independently that the most important peak among them was furfurylmethyl sulfide (XV), the compound described above. Identification was possible by MS interpretation. All these compounds show a typical isotope peak owing to [34]S at $M + 2$ (2); as the furfuryl fragment (m/e 81) was the most important peak in this MS, formula XV was suggested.

A second small peak indicated the presence of a methyl homolog (XVI)—in fact, its MS pattern parallels the one of XV quite closely; its parent peak is at m/e 142, and abundance of $M + 2$ distinctly indicates S. Base peak is at m/e 95—i.e., 81 + 14— and a rather strong peak appears at m/e 43 suggesting an acetyl fragment which originates from the α-methylfuran grouping. 5-Methylfurfuryl-2-methyl sulfide (XVI)

therefore was formulated as an hypothesis and proved by the synthesis of this compound, starting from 5-methylfurfural by known methods. GC and MS behaviors of the synthetic sulfide are in agreement with the natural product (XVI).

A third, small constituent of sulfury odor was eluted considerably later in GC. According to its MS, the molecular weight was 194, sulfur present (isotope peak at 196), and the most important fragment was m/e 81—i.e., furfuryl. Comparison of its MS with that of difurfuryl-ether (Table I) shows a striking analogy, and difurfuryl sulfide was suggested immediately. Synthesis of this product established structure XVII for the compound.

Two new types of furan-sulfur derivatives were encountered in XVIII and XIX, both isolated in very small amount. The lower boiling constituent showed the rather simple MS given in Table III.

The molecular weight of XVIII is 142 and the isotope peak at 144 shows the presence of sulfur. Base peak is at m/e 95—i.e., a methylfurfuryl or furoyl fragment is suggested. The rather stable nature of the molecule is indicated by the strong parent peak, and the occurrence of peak 39, being third in importance, means formation of $C_3H_3^+$ ion from the furan ring. Peak 45 shows loss of CHS originated presumably from a SCH_3 group, and peak 67 stems from fragment m/e 95 by loss of CO, thus excluding a methylfurfuryl fragment, which is also confirmed by absence of m/e 43. Interpretation of these results led to the formula of methylthiofuroate (XVIII), a thioester. This compound is prepared easily from furoylchloride and methylmercaptan, yielding synthetic XVIII, which proved to be identical with the natural product.

The second small peak showed the MS reproduced in Table III (there are impurities in the natural sample which could not be purified further because of the small amount available). Its molecular weight is 156,

Table III. Mass Spectral Data of Furan Thioesters Compared with Furfurylisopropyl Sulfide (FIS)

m/e	XVIII Synth.	XVIII Nat.	XIX Synth.	XIX Nat.	FIS Synth.
27		2.1	6.7	10.5	7
29	0.7	2.1		3.7	
37	1.5	2.1			
38	4.1	4.9			
39	15.9	21.1	2.8	5.9	
40		1.1			3.3
41		3.2		5.2	5.5
43		4.6	27	24.7	3.3
45	4.1	7.7	8.3	9.3	5.2
47	2.2	2.5			1.2
50				1.9	
51		1.4	2.8	3.7	
52	0.7		2	3.7	
53	0.7	1.1	17.5	19.1	10
54	0.7				
55		1.1			
57		0.7		3.1	
59					1.8
60		1.8			
67	3.3	3.5			
69			0.8	3.7	
74		1.1			
77		3.5			
81	0.7	1.4	100	100	100
82			5.2	6.2	5.5
83		2.1			
85			1.2	2.5	0.9
91		2.8			
95	100	100			
96	4.8	5.3			
98				2.5	
104		2.5			
105		5.3			
108		1.4			
109		1.4			
111		11.3			
113			3.6	2.8	0.9
114			3.6	3.1	
134		1.1			
136		1.8			
142	26.2	25.4			
143	2	1.8			
144	1.3	1.2			
156			16.3	12.3	21.8
157			1.4	1	2
158			0.8	0.6	1
165		1.8			
182				1 5	

presence of sulfur is indicated by the isotope peak at m/e 158, and base peak is the fragment 81. The presence of a homolog of furfurylmethyl sulfide was suspected first, judging from the MS pattern of XV. Furfurylpropyl and isopropyl sulfide were, therefore, prepared. The MS of these two sulfides is very similar and the one of furfurylisopropyl sulfide is shown in Table III. Despite close resemblance, it appears that XIX is characterized by an extra peak at m/e 43— i.e. an acetyl fragment. A thioacetate structure explained this difference nicely, assuming that XIX was furfuryl thioacetate. Preparation of this substance from furfurylmercaptan and acetyl chloride gave synthetic XIX; its MS and GC data are in agreement with those of natural XIX.

A last example in this class is represented by compound XX. Its MS is given in Table IV. Molecular weight is 128—i.e., an isomer of XV is present which has the same general formula, C_6H_8OS. Assuming it was a furan derivative, formula XX appeared very attractive; stability of such a compound is confirmed by the important parent peak being base peak. Second and third in importance are the fragments at m/e 113 and 85, respectively, indicating loss of methyl and acetyl, the latter causing rupture of the furan ring. Peak 45 presumably means formation of CHS^+ from the SCH_3 grouping (cf. methylthiofuroate, Table III)

and the small fragment at m/e 47 confirms this assignment. Synthesis of this product could be realized in small yield by mixing 5-methylfuran with the very reactive methylsulfenyl chloride in pyridine. 2-Methylthio-5-methylfurane produced by this procedure proved to be identical with natural XX. Very recently, further representatives of such furan sulfides have been described (10) by reaction of furyllithium with sulfur followed by an alkyl halide.

Miscellaneous. Among the products of a hydrocarbon-like subfraction, a product was isolated in very small amount which was eluted closely to naphthalene in GC and smelled similar. From its MS (Table V), the product had a molecular weight of 140 and contained two atoms of sulfur, based on the importance of the isotope peak at mass 142. From its occurrence and stability (MS), a structure related to an aromatic hydrocarbon was expected, and the formula $C_6H_4S_2$ actually leaves little speculation. Thiophene (XXI) suggested itself readily; it is formed in small yield by pyrolysis of citric acid in the presence of phosphorus and sulfur (1). Synthetic XXI shows the MS given in Table V, which is almost identical with the one of our product. Nevertheless, the isomeric 2,3,3',2'-thiophenothiophene (XXII) might behave slightly differently and explain these discrepancies. Synthesis according to literature (4) gave XXII, the MS (Table V) of which is almost indistinguishable from the one of XXI. Unfortunately, the natural product does contain some by-products and

Table IV. Mass Spectral Data of 2-Methylthio-5-methylfuran—Natural and Synthetic

m/e	XX Synth.	XX Nat.
27	7.3	8.8
39	2.8	3.1
41	3.1	4.1
43	11.1	11.9
45	17.1	19.1
47	1.8	1.5
50	3.4	3.1
51	5.7	5.7
52	2.9	2.1
53	6	6.2
59		2.1
64	1.3	1
65	1.6	1
69	7	6.7
70	5.7	4.6
71	1.8	1
81	1.8	2.1
85	41.2	42.3
86	1.8	1.5
87	1.3	1
98	1.6	1.1
113	72.5	72.7
114	3.9	3.1
115	2.9	1.9
128	100	100
129	7.6	7.3
130	4.8	4.6

Table V. Mass Spectral Data of Isomeric Thiophenothiophenes Compared with Natural Sample XXI or XXII

m/e	XXI Synth.	XXII Synth.	XXII Nat.
38	1.4	0.8	1
39			2.1
45	8.1	9.4	13.4
50			1.8
51			1.8
57	0.8	1.3	1.5
58	0.8	0.8	0.8
63	5.1	2.7	2.3
64	3.8	3	3.6
69	10.3	11.8	6.9
70	7.9	10.5	8.2
71	2.4	4.3	3.1
76	1.9		0.8
81	1.6	1.3	1.5
82	2.4	2.2	2.6
88		1.1	1
89			1.3
93	1.4	1.6	2.1
95	6.5	5.6	8.5
96	19.8	19.4	21.6
97	1.1	1.1	1.3
139	1.4	1.6	1.8
140	100	100	100
141	8.5	8.3	8.2
142	9.2	9.1	9.1

Table VI. Mass Spectral Data of 5-Methylquinoxaline (XXV), Natural and Synthetic, Compared with 2-Vinyl-benzofuran (XXIII) and 6-Methylquinoxaline (XXIV)

m/e	XXIII Synth.	XXIV Synth.	XXV Nat.	XXV Synth.	m/e	XXIII Synth.	XXIV Synth.	XXV Nat.	XXV Synth.
26		0.9	1.4	0.8	142		2.1	2.8	2.4
27	2.2	2.3	2.6	1.2	143	2.9	15.9	22.8	22.4
32		3			144	100	100	100	100
38	1.4	1.9	2.3	1.6	145	11	10.5	10.9	10.5
39	4.8	6.8	7	6.3					
40		1.4	1.4	1.2					
50	2.6	2.6	2.6	2					
51	3.6	3.5	3.7	2.8					
52		2.3	2.8	2					
57	3.3								
57, 5	3.8								
58	3.3								
58, 5			3.5	3.2					
62	3.1	3	3.3	2.8					
63	7.7	8.6	9.8	9.5					
64	1.7	5.1	6	5.5					
65	1.9	1.6	2.3	1.6					
72	6.5	4.7							
74	1.2		0.9						
75	1.2	1.2	1.9	1.2					
76		1.2	1.6	1.2					
77			1.2	0.8					
86	1.2								
87	1.4								
88	1.2								
89	6.2	18.7	16.7	16.1					
90	1.4	31.8	28.6	28					
91		2.6	3.3	2.4					
114	1.9								
115	42.6								
116	5.3	3	4.2	3.2					
117		16.8	14.4	13.4					
118	1.9	1.9	2.6	2					

no further purification was possible. Retention time on different columns could not be checked either. The available information shows that one of the 2-thio-phenothiophenes undoubtedly is present in coffee; no clear decision as to which one could be reached in this case.

A nitrogen-containing product was found in a sub-fraction of medium polarity including p-vinylguaiacol. On elution from GC it smelled like a mono- or dimethyl-pyrazine but not much attention was paid to this fact at first. The MS of this constituent is shown in Table VI. No similar pattern could be found among the substances isolated, and a general formula of $C_{10}H_8O$ was derived as a hypothesis. On checking the literature, 2-vinyl-benzofuran (XXIII) was found to fit the available data reasonably well. Benzofuran as well as 2-methyl-benzofuran had been identified in another fraction, supporting this hypothesis. 2-Vinylbenzofuran there-fore was prepared, starting from 2-acetylbenzofuran; reduction to the corresponding carbinol and pyrolysis of its acetate produced this product in a rather simple manner. Table VI shows, however, that its MS clearly

differs from the one of the natural substance. Close examination of the latter prompted revision of its formula to $C_9H_8N_2$, remembering the information given by its odor. The presence of a pyrazine is suggested immediately by this formula. Pyrazines have been known in coffee for a long time (15) and probably are formed during roasting by interaction of amines, derived from protein degradation, with carbohydrates. This information led to a methylquinoxaline which looked very attractive and compatible with the MS pattern required. There was no indication which isomer was present. 2-Methylquinoxaline was syn-thesized first. Its MS showed all the major fragments corresponding to those of natural XXV—however, of markedly different intensity. The isomeric 6-methyl-quinoxaline (XXIV) therefore was prepared next. Its MS is reproduced in Table VI. It is almost identical with that of the coffee constituent.

The chance that the latter, however, would cor-respond to the third possible isomer was not excluded. 5-Methylquinoxaline (XXV) was synthesized con-sequently and proved to be identical with natural XXV.

Relative intensities of all fragments in the MS are well in agreement; the only major differences between XXIV and XXV consist in the absence of a small fragment at m/e 72 as well as in gain of importance of the $M-1$ peak at m/e 143 in the latter.

Acknowledgment

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