accompanying naringenin rutinoside. Compound III failed to crystallize, even when seeded with crystalline 2''-*O*-β-D-xylosylvitexin obtained from *Vitex lucens*. It was freely soluble in water. Compound III was indistinguishable from 2''-*O*-β-D-xylosylvitexin on paper chromatography in a variety of solvents or on paper electrophoresis (Table II). The two compounds were also indistinguishable (R_f = 0.18) on polyamide tlc using nitromethane–methanol (2:1).

Hydrolysis of 2''-*O*-β-D-Xylosylvitexin (III).—A sample of compound III in aqueous 2 *N* hydrochloric acid was heated on the steam bath for 30 min. The solution was extracted with ethyl acetate. Evaporation of the extract afforded a mixture of vitexin and isovitexin, as shown by paper chromatography and electrophoresis. When the hydrolysis was carried out enzymatically at pH 4.6 using crude hemicellulase,[28] the ethyl acetate extract contained mainly vitexin together with a very small proportion of isovitexin. The presence of xylose in the aqueous layers remaining from these hydrolyses was demonstrated by paper chromatography.

Registry No.—I, 15822-81-8; Ia, 15895-78-0; II, 15822-82-9; IIa, 15895-77-9; III, 11044-10-3; VI, 520-36-5; VIa, 3316-46-9; VII, 491-70-3; VIIa, 1061-93-4; VIII, 491-71-4; VIIIa, 3162-04-7; IX, 520-34-3; IXa, 3162-05-8; X, 1397-60-0; Xa, 11040-83-8; XI, 11044-04-5; XIa, 11044-05-6; XIVa, 11044-08-9; XVa, 11044-03-4; XVIIa, 11044-09-0; XXIIa, 11044-06-7; XXVa, 6980-38-7; XXVIa, 11044-07-8; XXXIIa, 5892-39-7; D-glucose 2,3,4,6-tetraacetate, 10343-06-3.

Dihydroisocoumarins from a *Sporormia* Fungus

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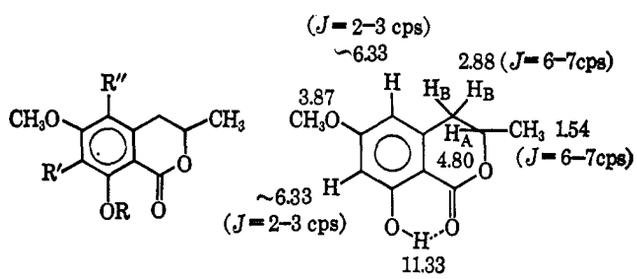
Three dihydroisocoumarins, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, 5,7-dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, and 7-chloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, have been isolated from a *Sporormia* fungus. The structures of the two new chlorinated dihydroisocoumarins have been established by spectral studies and by chemical conversion.

Sondheimer[1] isolated 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (I) from carrots which had developed a bitter taste during storage. Condon, *et al.*,[2-4] later associated the production of this fungitoxic substance in carrots with alterations in the normal metabolism of the carrot root tissue which they felt were possibly induced by the presence of fungi. Recently, Aue, *et al.*,[5] have reported the isolation of this same compound, which is sometimes referred to as 6-methoxymellein, from a submerged culture of the fungus *Sporormia bipartis* Cain. In our work on the metabolic products of *Sporormia affinis* Sacc., Bomm and Rouss, we have isolated not only 6-methoxymellein (I) but also the closely related halogenated compounds 5,7-dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (IV) and 7-chloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (V).

These findings and those of Aue, *et al.*,[5] suggest to us that the occurrence of I in fungal infected carrots might be due to the fungus itself. The presence of I in two *Sporormia* species may also be noteworthy in a chemotaxonomic sense.

For our purposes, the fungus *Sporormia affinis* was grown in submerged culture under standard conditions and the metabolic products were isolated after 120 hr by carbon adsorption followed by chromatography. A major product was identical in its physical and chemical properties with 6-methoxymellein; its identity was confirmed by comparison with an authentic specimen.[1]

In determining the structure of the two minor chlorinated metabolites, the nmr and mass spectra of I were quite revealing and it is appropriate to discuss them at this stage. The various peaks in the nmr spectrum are assigned pictorially in formula VIII.



VIII

I, R = R′ = R″ = H
II, R = CH_3; R′ = R″ = H
III, R = CH_3; R′ = R″ = Cl
IV, R = H; R′ = R″ = Cl
V, R = R″ = H; R′ = Cl
VI, R = CH_3; R′ = Cl; R″ = H
VII, R = CH_3; R′ = H; R″ = Cl

The three-proton doublet at 1.54 ppm (J = 6–7 cps) is attributed to the methyl group on the carbon bearing a single proton and attached to the electronegative oxygen. A doublet at 2.88 ppm is assigned to the virtually equivalent methylene protons H_B split by the single proton H_A of the asymmetric center. A sharp three-proton singlet at 3.87 ppm is due to the methoxy group and the one-proton multiplet at 4.80 ppm arises from the coupling of the methyl and methylene group with the single proton H_A. The aromatic region contains split signals (J = 2–3 cps typical for *meta*-coupled protons) for two barely separated protons at about 6.33 ppm and the exchangeable proton of the intramolecularly bonded hydroxyl group is observed at 11.33 ppm. The mass spectrum confirms the molecular weight by a peak at m/e 208 and contains a number of other significant peaks. The most abundant peak at m/e 164 (400%) arises because of loss of acetaldehyde. A metastable peak at 129.3 mass units confirms this loss from the molecular ion. The loss of $CH_3CO\cdot$ accounts for the peak at m/e 165 (100%). Elimination of CO from the molecular ion of I occurs as is evidenced by m/e 180 (9%) but is clearly not a

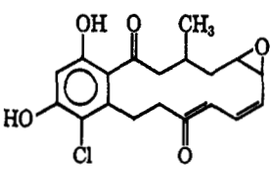
(1) E. Sondheimer, *J. Amer. Chem. Soc.*, **79**, 5036 (1957).
(2) P. Condon and J. Kuc, *Phytopathology*, **50**, 267 (1960).
(3) P. Condon and J. Kuc, *ibid.*, **52**, 182 (1962).
(4) P. Condon, J. Kuc, and M. H. Draudt, *ibid.*, **53**, 1244 (1963).
(5) R. Aue, R. Mauli, and R. P. Sigg, *Experientia*, **22**, 575 (1966).

significant feature.[6] Expulsion of CO from this system seems to be largely replaced by a fragmentation where 29 mass units (CHO) are removed to give the peak *m/e* 179 (49%). Our data are suggestive that the hydrogen of this fragment does not seem to come, as might be expected, entirely from the phenolic group, but may in part also come from the adjacent aromatic position. When the adjacent proton is replaced by chlorine, as in the case of the other metabolites, the loss of CHO is roughly halved while loss of elements COCl is then observed in about the same abundance. On the other hand, the data so far available does not rule out the sequential loss of CO and Cl.

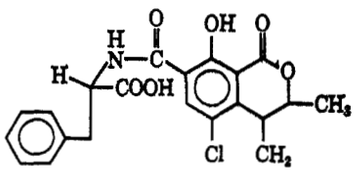
The second compound isolated from the *Sporormia* fermentation melts at 225–226° and has a molecular formula of $C_{11}H_{10}O_4Cl_2$ (compound IV). The material is optically active and has low solubility in ether but is readily soluble in ethyl acetate, chloroform, acetone, and the lower alcohols. It can be extracted from its chloroform solution by 5% aqueous bicarbonate solution and gives a positive ferric chloride test. It does not form a methyl ether with diazomethane but it is converted into this derivative by using a mixture of sodium hydroxide and dimethyl sulfate. In the infrared spectrum of the dichloro compound a carbonyl peak is observed at 1695 cm^{-1}, whereas in the methyl ether of the material, this carbonyl peak is shifted to 1737 cm^{-1}. These features are consistent with the behavior of a substance containing a chelated phenolic group.[7] The ultraviolet spectrum of compound IV shows peaks at 224, 260, and 310 mμ. In alkaline solution the low wavelength absorption undergoes a bathochromic shift to 244; the peak at 260 is obliterated while a large hyperchromic effect is observed at 310 mμ. These changes are reversed upon neutralization of the alkaline solutions. The ultraviolet absorption characteristics of the compound were strongly reminiscent of those of I and the close relationship between the two compounds was further confirmed by nmr spectral data.

The nmr spectrum of the dichloro metabolite contains a doublet at 1.55 ppm (J = 6–7 cps) assignable to a methyl group split by a single proton. Instead of a doublet at 2.88 as is observed in the spectrum of I there is a multiplet centered at 2.95 ppm. In the chloro compound the nonequivalence of the methylene protons is enhanced because of the *peri* effect of the chlorine atom at the 5 position in the aromatic ring. The three-proton signal due to the methoxy group appears at 3.92 ppm exactly as with 6-methoxymellein. The single proton of the asymmetric center now gives rise to a complex multiplet at 4.58 ppm. The extra splitting in this case, as opposed to the much less complicated multiplet at 4.80 ppm in the spectrum of I, again arises because of the enhanced nonequivalence of the methylene protons in the dichloro metabolite.

A similar nonequivalent benzylic methylene group was observed by McCapra, *et al.*,[8] and Mirrington and others[9] in their work on monorden and by Van den Merwe and his group in their identification of the



monorden



ochratoxin A

ochratoxins.[10] The mass spectrum of IV, while not as definitive as that of I, nevertheless substantiates the chlorinated dihydroisocoumarin structure. The molecular ion peak at 276, 83% (*m/e* 215 taken as 100%) together with peaks at 278 (56%) and 280 (10%) are in good agreement with Beynon's postulates for doubly chlorinated compounds.[11] Large significant peaks at 233 (38%) and 232 (82%) are attributable to the loss of $CH_3CO\cdot$ and acetaldehyde which are characteristic fragments of the 3-methyldihydroisocoumarin structure. No peak is observed for the loss of CO but there are fragments representing the loss of CHO *m/e* 247 (16%) and COCl *m/e* 213 (14%).

The dechlorination of the aromatic ring of tetracycline compounds by catalytic hydrogenation is well known.[12] It was felt that this technique might be used to convert the chlorinated metabolite into 6-methoxymellein after first protecting the phenolic group as the methyl ether. However, in our hands, this approach did not prove fruitful. Another procedure, that of converting the methyl ether of 6-methoxy mellein (II) into the corresponding dichloro derivative III by direct chlorination was successful. Whalley's method,[13] using a mixture of sulfuryl chloride and aluminum chloride, converted II into a monochlorinated derivative which we obtained in small quantity and believe is the 5-chloro compound VII. An older procedure, using sulfuryl chloride together with a solution of aluminum chloride in sulfur monochloride[14] resulted in the chlorination of the two open positions of the aromatic ring. The product has an identical melting point and infrared spectrum with those of the methyl ether III of the natural product.

The monochlorinated metabolite isolated from *S. affinis* fungus is a white, optically active, crystalline material which melts at 169–170° and has the empirical formula $C_{11}H_{11}O_4Cl$. The material is sparingly soluble in ether and in 5% bicarbonate solution but readily soluble in ethyl acetate, chloroform, and the lower alcohols and gives a positive ferric chloride test. The ultraviolet spectrum in methanol has peaks at 224, 272, and 305 mμ. In alkaline solution the lower absorbance peak undergoes a red shift to 230 mμ; the peak at 272 mμ exhibits a hypsochromic effect while the third absorbance maximum is shifted to 340 mμ and in addition displays a hyperchromic effect. As in the case of the other two metabolites, all of these changes are reversed upon acidification.

The infrared spectrum shows a strong carbonyl absorption at 1645 which is shifted to 1720 cm^{-1} upon

(6) J. P. Kutney, G. Eigendorf, D. L. Dreyer, and L. A. Mitscher, *Can. J. Chem.*, in press.

(7) T. M. Meijer and H. Schmid, *Helv. Chim. Acta*, **31**, 1603 (1948).

(8) F. McCapra, A. I. Scott, P. Delmotte-Plaquee, and N. S. Bhacca, *Tetrahedron Lett.*, 869 (1964).

(9) R. N. Mirrington, E. Ritchie, C. W. Shoppe, S. Sternhall, and W. C. Taylor, *Aust. J. Chem.*, **19**, 1265 (1966).

(10) K. J. Van den Merwe, P. S. Steyn, and L. Tourie, *J. Chem. Soc.*, 7083 (1965).

(11) J. H. Beynon, "Mass Spectrometry and Its Application to Organic Chemistry," Elsevier Publishing Co., Amsterdam, 1960, p 298.

(12) C. R. Stephens, L. H. Conover, R. Pasternak, F. A. Hochstein, W. T. Morland, P. P. Regna, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Amer. Chem. Soc.*, **76**, 3568 (1956).

(13) J. S. E. Holker, W. J. Ross, J. Staunton, and W. B. Whalley, *J. Chem. Soc.*, 4150 (1962).

(14) O. Silberrad, *ibid.*, 1015 (1922).

formation of the methyl ether. The similarities in the ultraviolet and infrared spectra of the three metabolites indicated that all three are closely related structurally. It was felt that the third product had the 3-methyldihydroisocoumarin structure with a chlorine atom in either the 5 or the 7 position. The nmr spectral data clearly indicated that the chlorine is in the 7 position and, hence, the structure of the product is 7-chloro-3-methyl-6-methoxy-8-hydroxy-3,-4-dihydroisocoumarin (V). The nmr spectrum of V is identical with that of 6-methoxymellein (I) except in the aromatic region where V exhibits a one-proton singlet at 6.33 ppm. The methylene protons of V are virtually equivalent as in I demonstrating the lack of a *peri* effect and, hence, the chlorine atom cannot occupy the 5 position.

The mass spectrum of V has large peaks at *m/e* 199 (72%) and 198 (108%) (*m/e* 242 taken as 100%) accounted for by loss of $CH_3CO\cdot$ and CH_3CHO, respectively. Presence of metastable peaks at approximately 162 and 146 mass units confirm the acetaldehyde fragmentation. The former accounts for the loss of CH_3CHO from the molecular ion and the latter arises because of the loss of water from the resultant fragment (198 − 18) → 180 = 146. The excision of CHO *m/e* 213 (16%) and COCl *m/e* 179 (16%) is also noted as in the case of the dihalo compound.

Some difficulty was encountered in forming the methyl ether of V. Use of dimethyl sulfate and sodium hydroxide failed to methylate this material although these reagents worked satisfactorily with compounds I and IV. Refluxing of the material in acetone with iodomethane and sodium carbonate[15] also failed to effect the desired reaction. The methyl ether was prepared by the method of Garden and Thomson[16] using silver oxide and methyl iodide in chloroform. All three metabolites exhibited low potency antifungal activity.

Experimental Section

Nmr spectra were run on a Varian A60 instrument under normal conditions. Mass spectra were run on an AE I MS9 high-resolution, direct-inlet mass spectrometer.

3-Methyl-6-methoxy-8-hydroxy-3,4-dihydroisocourmarin (I).—*Sporomia affinis* Sacc., Bomm and Rouss (Lederle culture N313), was deep-fermented using standard conditions of agitation and aeration for 120 hr at 28° on a medium consisting of 2.0 g of molasses, 1.5 g of corn starch, 1.0 g of cerelose, 0.75 g of soya peptone, 0.5 g of calcium carbonate, and 0.25 g of prograsol[17] per liter of water. The whole mash was filtered and filtrate was treated with 10% w/v of charcoal. The charcoal pad was eluted with acetone–water (90:10) at pH 2.0 and the eluate concentrated to the aqueous phase which was extracted with chloroform. The chloroform extracts were concentrated to a gum which was chromatographed over silica gel (Davidson Grade 923) using chloroform–hexane (1:1) as developing solvent. The less polar fraction from the adsorption column was partitioned over diatomaceous earth using the partitioning system hexane–ethyl acetate–methanol–water (85:15:15:6). The material eluting in the second holdback volume was recrystallized from ether–hexane to obtain I in yields of up to 22 mg per liter of mash: mp 75.5–76°; $[\alpha]^{25}D$ −51.0 ± 3.0 (*c* 1.50 MeOH) (lit.[1] mp 75–76°; $[\alpha]^{24}D$ −56.0 [*c* 1.0, MeOH]); λ_{max} (MeOH) 302 mμ (ε 4890), 267 (12,580), and 216 (19,860); ν_{max} (KBR) 1665, 1630, 1580, 1375, 1245, 1205, 1160, 1115, 1090, 1070, 1038, 965, 850, 828, 800, and 707 cm^{-1}; nmr ($CDCl_3$) at δ 1.54 ($-CH_3$, doublet, *J* = 6–7 cps), 2.88 ($-CH_2-$, doublet, *J* = 6–7 cps), 3.87 ($-OCH_3$, singlet), 4.80 (>CH–, multiplet), 6.33 (aromatic 2 H, split singlets, *J* = 2–3 cps), and 11.33 (–OH, exchangeable singlet).

Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; mol wt, 208. Found: C, 63.30; H, 5.69; mol wt, 208 ± 0 (mass spectroscopy).

3-Methyl-6,8-dimethoxy-3,4-dihydroisocoumarin (II).—The methyl ether of I was prepared as described by Sondheimer[1] in 50% yield following recrystallization from ethyl acetate–hexane: mp 125–126°; $[\alpha]^{25}D$ −152 ± 2.8° (*c* 1.05); λ_{max} (MeOH) 297 mμ (ε 6210), 263 (13,540), and 214 (23,530); ν_{max} (KBR) 1710, 1600, 1463, 1343, 1250, 1198, 1163, 1115, 1084, 1043, 855, and 790 cm^{-1}; nmr ($CDCl_3$) at δ 1.42 ($-CH_3$, doublet, *J* = 6–7 cps), 2.80 ($-CH_2-$, doublet, *J* = 6–7 cps), 3.83 ($-OCH_3$, singlet), 3.90 ($-OCH_3$, singlet), 4.45 (>CH–, multiplet), 6.33 (aromatic 2 H, split singlets, *J* = 2–3 cps).

Professor Sondheimer was kind enough to forward us a sample of the methyl ether of 6-methoxymellein. The melting point and infrared curve of this material were identical with those of II.

5,7-Dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (IV).—This metabolite was obtained by following the isolation procedure described for I up to the point of extraction of the concentrated charcoal eluate with chloroform. The chloroform extract was back extracted with 10% sodium bicarbonate solution. The bicarbonate extract was acidified and extracted three times with chloroform. The chloroform extracts were dried over anhydrous magnesium sulfate and concentrated to an oil which was allowed to stand at room temperature for 3–4 days, during which time a solid formed. Trituration of the oil–solid mixture with a little ether gave a suspension which could be filtered to get the solid residue. Recrystallization from ethyl acetate–hexane yielded IV: mp 225–226°; $[\alpha]^{25}D$ −142.0 ± 2.8 (*c* 1.067, MeOH); λ_{max} (MeOH) 310 mμ (ε 5540), 260 (7890), and 224 (24,830); λ_{max} (methanolic 0.1 *N* NaOH) 310 mμ (ε 28,060) and 244 mμ (ε 17,300); ν_{max} (KBR) 1695, 1575, 1425, 1410, 1355, 1260, 1115, 1095, 960, 798, 790, 775, and 738 cm^{-1}; nmr ($CDCl_3$) at δ 1.55 ($-CH_3-$ doublet, *J* = 6–7 cps), 2.88 ($-CH_2-$, multiplet), 3.92 ($-OCH_3$, singlet), 4.58 (>CH–, multiplet).

Anal. Calcd for $C_{11}H_{10}O_4Cl_2$: C, 47.65; H, 3.63; O, 23.10; Cl, 25.63; mol wt, 277. Found: C, 48.05; H, 3.44; O, 24.15; Cl, 24.95; mol wt, 276 ± 0 (mass spectroscopy).

Yields of the crystalline product were of the order 0.5–1.0 mg per liter of mash. In cases where the amount of IV present was small and hence it failed to crystallize after 3–4 days as described, it was necessary to pass the oil over silica gel. Elution was carried out using chloroform–hexane (50:50) and the material which was recovered from the fifth through seventh holdback volumes was partitioned over diatomaceous earth using hexane–ethyl acetate–methanol–water (85:15:15:6). Compound IV was eluted in the first three to four holdback volumes, whereas V came off in the eighth through eleventh volumes. This was a little unexpected since IV is a stronger acid than V and hence should be a more polar material.

5,7-Dichloro-3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin (III). A. Chlorination of 3-Methyl-6,8-dimethoxy-3,4-dihydroisocoumarin.—In our first attempt to chlorinate II we followed Whalley's procedure.[9] To a solution of 150 mg (0.67 mmol) of 3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin (II) in 20 ml of carbon tetrachloride 0.1 ml of sulfuryl chloride was added followed by 100 mg of aluminum trichloride. The mixture was allowed to stand at room temperature overnight. Work-up yielded a yellowish oil which was passed over 10 g of acid-washed silica gel by elution with ethyl acetate–hexane (20:80). The material which came off in first and second holdback volumes was recrystallized from ethyl acetate–hexane to get 25 mg of product, mp 119–120°, which gave the elemental analysis of a monochlorinated compound: ν_{max} (KBR) 1725, 1598, 1460, 1425, 1375, 1230, 1205, 1107, 1050, 925, 802, 772, and 750 cm^{-1}.

Anal. Calcd for $C_{12}H_{13}O_4Cl$: C, 56.14; H, 5.07; Cl, 13.83. Found: C, 56.70; H, 5.11; Cl, 13.74.

It is likely that this material is the compound 5-chloro-3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin as it is isomeric with product VI, although this conclusion has not been verified.

Using the method of Silberrad[10] it was possible to obtain the desired product in good yield. The chlorinating agent, consisting of 1 ml of sulfuryl chloride together with 0.1 ml of sulfur monochloride and 10 mg of aluminum trichloride, was first prepared to give a reddish mixture. To this, 100 mg (0.45 mmol) of the

(15) G. A. Ellestad, H. A. Whaley, and E. L. Patterson, *J. Amer. Chem. Soc.*, **88**, 4109 (1966).

(16) J. F. Garden and R. H. Thomson, *J. Chem. Soc.*, 2483 (1957).

(17) Distillers grain solubles from corn; Publicker Industries, Inc., Philadelphia, Pa.

methyl ether of 6-methoxymellein (II) were added directly. The crystals dissolved instantly with the evolution of gas bubbles. The resulting red solution was evaporated under reduced pressure to an oil–solid mixture which was triturated with ether and about 40 mg of a water-soluble solid were filtered off. The ether solution was concentrated to an oil and chromatographed over 10 g of acid grade Woelm alumina and eluted with ethyl acetate–hexane (1:10). The material obtained from the first holdback volume weighed 95 mg. After several recrystallizations from ether–hexane and ethyl acetate–hexane, 50 mg of white product was obtained: mp 81.5–82°; $[\alpha]^{25}D$ −160 ± 2.9 (*c* 1.037, MeOH); λ_{max} (MeOH) 305 mμ (ε 1740), 252 (6530), and 220 (33,350); ν_{max} (KBR) 1735, 1565, 1455, 1405, 1377, 1337, 1250, 1125, 1096, 1050, 980, 955, 930, 793, 765, and 747 cm^{-1}; nmr ($CDCl_3$) at δ 1.55 (–CH_3, doublet, *J* = 6–7 cps), 2.92 (–CH_2–, multiplet), 3.97 (–OCH_3, singlet), 4.00 (–OCH_3, singlet), 4.50 (>CH–, multiplet).

Anal. Calcd for $C_{12}H_{12}O_4Cl_2$: C, 49.48; H, 3.78; Cl, 24.39. Found: C, 50.10; H, 3.96; Cl, 24.44.

B. Methylation of 5,7-Dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin.—Approximately 40 mg (0.15 mmol) of IV were added to 0.3 ml of dimethyl sulfate, and 4 *N* sodium hydroxide was added dropwise until the mixture gave an alkaline reaction. The suspension was heated on a steam bath for 15 min. Tlc on Eastman sheets (type K301R) using hexane–ethyl acetate (60:40) indicated that the methyl ether had formed. The solution was acidified with dilute HCl and extracted with ether. The ether extracts were dried over anhydrous magnesium sulfate and concentrated to an oil which was chromatographed over 7.5 g of silica gel to give a nonpolar fraction which, on recrystallization from ethyl acetate–hexane, yielded 20 mg of product, mp 81.5–82°. The infrared spectrum of this material was identical with that of the product obtained by method of Silberrad. The congruency of these materials is conclusive proof of the identity of this metabolite since 6,8-dimethoxymellein is known.

7-Chloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (V).—Submerged fermentation of the *S. affinis* as described under the isolation of I was handled in the manner described up to the point of extraction of the concentrated charcoal eluate. The eluate was extracted with ether and the combined extracts were concentrated to an oil which was chromatographed over acid-washed silica gel and eluted with chloroform–hexane (75:25). The material eluting in the seventh through twelfth holdback volumes was partitioned over diatomaceous earth using the system hexane–ethyl acetate–methanol–water (85:15:15:6). The material which came off in the first and second holdback volumes consisted of a mixture of I and V. The two materials were then separated cleanly by chromatography over silica gel using hexane–ethyl acetate (95:5) as eluting solvent. Compound I was obtained from the fifth through seventh holdback volumes; V was recovered from the eleventh through thirteenth holdback volumes. Following recrystallization from ethyl acetate–hexane the yields of crystalline product V were in the range 0.0–0.5 mg per liter of mash: mp 170–171°; $[\alpha]^{25}D$ −71.3 ± 5.9 [*c* 0.505, MeOH]; λ_{max} (MeOH) 305 mμ (ε 680), 272 (12,600), and 224 (25,400); λ_{max} (methanolic 0.1 *N* NaOH) 340 mμ (ε 6420), 272 (25,000), and 224 (29,700); ν_{max} (KBR) 1645, 1565, 1512, 1423, 1380, 1325, 1285, 1265, 1210, 1205, 1150, 1120, 1093, 1030, 935, 908, 833, 803, 784, 760, and 700 cm^{-1}; nmr ($CDCl_3$) at δ 1.50 (–CH_3, doublet, *J* = 6–7 cps), 2.88 (–CH_2–, doublet, *J* = 6–7 cps), 3.97 (–OCH_3, singlet), 4.70 (>CH–, multiplet), 6.33 (aromatic 1 H, singlet), and 11.17 (–OH, exchangeable singlet).

Anal. Calcd for $C_{11}H_{11}O_4Cl$: C, 54.43; H, 4.53; Cl, 14.64; mol wt, 242.5. Found: C, 54.48; H, 4.95; Cl, 14.68; mol wt, 242 ± 0 (mass spectroscopy).

7-Chloro-3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin (VI).—Attempts to methylate V using dimethyl sulfate and sodium hydroxide solution failed. Since IV was methylated by this procedure no trouble had been anticipated. Approximately 70 mg (0.29 mmol) of V were dissolved in 0.5 ml of dimethyl sulfate and 4 N sodium hydroxide was added dropwise until the reaction mixture gave an alkaline reaction. The mixture was then heated on the steam bath for 15 min and allowed to sit overnight at room temperature. Work-up of the suspension yielded a solid which was chromatographed over silica gel, and eluted with chloroform to yield 40 mg of material, mp 130–131.5° with a trace persisting to 135°. The infrared curve of this material indicated that the isocoumarin ring had suffered some decomposition. Refluxing of V in acetone with anhydrous sodium carbonate and an excess of iodomethane for several hours[14] resulted, upon work-up, in recovery of starting material. The best method for the methylation of these chelated phenolic compounds appears to be that of Garden and Thomson.[15] To a solution of 40 mg (0.16 mmol) of V in 2 ml of chloroform approximately 100 mg of moist silver oxide were added together with 2 ml of iodomethane and the suspension stirred at room temperature for 1 hr. Tlc and ferric chloride testing at this stage showed the reaction to be incomplete and another 100 mg of silver oxide and 2 ml of iodomethane were added and stirring was continued for another hour by which time reaction was finished. The silver oxide was filtered off to give a colorless solution which on concentration yielded white crystals. Recrystallization from ethyl acetate–hexane gave 35 mg: mp 160.5–161.5°; $[\alpha]^{25}D$ −137 ± 10° (*c* 0.300, MeOH); λ_{max} (MeOH) 265 mμ (ε 12,610) and 220 mμ (ε 24,460); ν_{max} (KBR) 1720, 1595, 1450, 1408, 1370, 1353, 1310, 1262, 1216, 1206, 1185, 1117, 1105, 1064, 990, 933, 912, 858, 798, and 778 cm^{-1}; nmr ($CDCl_3$) at δ 1.45 (–CH_3, doublet, *J* = 6–7 cps), 2.83 (–CH_2–, doublet, *J* = 6–7 cps), 3.95 (2 × –OCH_3, singlet), 4.47 (>CH–, multiplet), and 6.63 (aromatic 1 H, singlet).

Anal. Calcd for $C_{12}H_{13}O_4Cl$; C, 56.14; H, 5.07; Cl, 13.83. Found: C, 56.46; H, 5.02; Cl, 13.71.

Registry No.—I, 13410-15-6; II, 15766-71-9; III, 15815-77-7; IV, 15815-78-8; V, 15815-79-9; VI, 15815-80-2; VII, 15815-81-3.

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