previously:[1] yield 550 mg (77.5% based on **20**): UV (0.1 N NaOH) λ_{max} 367 nm (ϵ 5416), 259 (29 204); UV (0.1 N HCl) λ_{max} 335 nm (ϵ 7677), 242 (22 000); NMR (TFA) δ 8.96 (s, 1 H, pteridine), 7.85 and 7.05 (2 d, 4 H, aromatic), 4.6 and 3.6 (t, t, 4 H, ethylene bridge); 4.01 (t, 1 H, α proton of glutamic acid), 2.05–2.85 (c, 4 H, glutamic acid). Compound **1** was also prepared using the procedure which was employed for the conversion of **21** to **2**. Anal. ($C_{20}H_{19}N_7O_6 \cdot H_2O$) C, H, N, O.

Methods Used for Biological Evaluation. Microbiological assays using the different strains of *Lactobacillus casei* and *Streptococcus faecium* were carried out as described previously.[1,27] Thymidylate synthase assays were performed according to the procedure of Friedkin.[28] Methods involving dihydrofolate reductase were also described previously.[29] The antitumor data on all compounds were collected under the auspices of the National Cancer Institute using the standard protocol (Instruction 14) for evaluating methotrexate analogues in the L-1210 lymphoid leukemia test system in mice (treatment schedule QD 1D × 09). Detailed procedures regarding the transport of some of these analogues in L-1210 and Ehrlich cells in culture, as well as procedures used to evaluate the growth inhibition potency of the analogues in the L-1210 cells, have been published recently from one of these laboratories.[26,30]

Acknowledgment. This investigation was supported by grants from the American Cancer Society [CH-53C (M.G.N.); CH-26 (F.M.S.)] and the National Institutes of Health [CA-27101 (M.G.N.); CA-10914 (R.L.K.); CA-18856, CA-22764 (F.M.S.) from the National Cancer Institute]. We are also indebted to Dr. Jacqueline Plowman and Robert B. Ing of the National Cancer Institute for their assistance in obtaining the antitumor data.

(27) Kisliuk, R. L.; Strumpf, D.; Gaumont, Y.; Leary, R. P.; Plante, L. *J. Med. Chem.* **1977** *20*, 1531.
(28) Wahba, A. J.; Firedkin, M. *J. Biol. Chem.* **1962**, *237*, 3794.
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Antimicrobial 3-Methyleneflavanones

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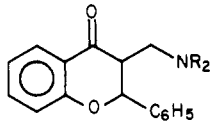
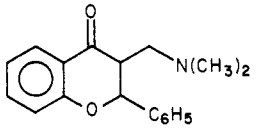
The antimicrobial activity previously attributed to flavanone Mannich bases was found to be due to their breakdown products, 3-methyleneflavanones. Among the latter compounds, highest potency was observed when the flavanone phenyl ring contained bromine or chlorine substituents. 3-Methylene-2-phenylflavanone (**8**) was synthesized and shown to be equal to hexachlorophene in tests against representative Gram-positive microorganisms.

In recent years two reports have appeared attributing in vitro antifungal and antibacterial activity to the Mannich bases (I) of flavanones.[1,2] However, although the



I



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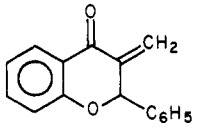

2

synthesis of several dozen analogues of I were reported, no clear structure–activity pattern was apparent. For example, bromo and methyl substituents on the benzo ring often affected activity in the same way; however, if they were attached to the phenyl ring, their effects were opposed. Analogues of I having cycloalkyl substituents on nitrogen displayed high activity, while aliphatic substituents of similar lipophilicity were inactive. Nevertheless, because of the potential usefulness of lipophilic analogues of I as topical antifungal agents, we reinvestigated the chemistry and biological activity of flavanone Mannich bases and their derivatives.

We first examined 3-[(dimethylamino)methyl]flavanone (**1**) which had been reported to be both active and inactive. Although this compound could be prepared as the HCl salt by the reaction of flavanone with formaldehyde and dimethylamine, its purification was difficult. Furthermore, when converted to the free base, **1** decomposed extensively on standing, even in nonpolar solvents. When a carefully purified sample of the HCl salt of **1** was neutralized with aqueous $NaHCO_3$ solution, extracted into ether, dried, and evaporated, 3-methyleneflavanone (**2**) was isolated in good yield. In our hands, the HCl salt of **1** displayed potent antimicrobial activity, and, when tested against 14 microorganisms, **2** possessed the same potency and spectrum of antimicrobial activity as purified **1**.

The Mannich bases appear to act as prodrugs for their unsaturated derivatives. Their instability probably explains the inconsistent structure–activity pattern observed earlier. Different substituents on nitrogen and inconsistencies of handling may have led to varying degrees of conversion of the bases to the corresponding 3-methyleneflavanones. Support for this view was obtained by synthesizing 3-[(dimethylamino)methyl]-3-methylflavanone (**3**). This compound, which cannot eliminate dimethylamine, was inactive when tested against 22 fungi and bacteria.

(1) J. J. Gavin, H. H. Walchli, and D. A. Stauffer, U.S. Patent 3 753 985, Aug 21, 1973.
(2) G. M. Cigolani, F. Gualtieri, and M. Pigini, *Farmaco, Ed. Sci.*, **26**, 718 (1971).

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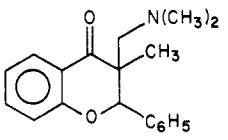

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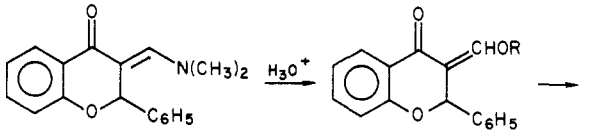
Having established the 3-methylene compounds **2** as the biologically active flavanone species, we prepared three types of analogues: those having substituents in the phenyl and the benzo rings and those substituted at the methylene function.

Chemistry. Phenyl- and benzo-substituted 3-methyleneflavanones were prepared either by decomposing the appropriate Mannich bases under alkaline conditions or by reaction of the flavanones directly with bis(dimethylamino)methane (see Table I).[3]

Four 3-methyleneflavanones having substituents on the methylene group were prepared by first reacting flavanone itself with dimethylformamide dimethyl acetal to give 3-[(dimethylamino)methylene]flavanone (**4**). This was



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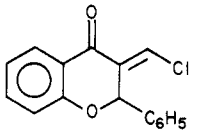
5, R = H
6, R = CH_3SO_2



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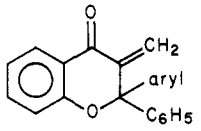
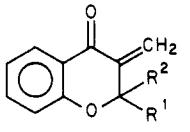
hydrolyzed to 3-(hydroxymethylene)flavanone (**5**). The reaction of **5** with methanesulfonyl chloride gave the enol mesylate **6**, which, upon treatment with powdered NaCl and 18-crown-6, produced the chloro ketone **7** in good yield.

In addition to the methyleneflavanones mentioned above, several miscellaneous analogues were synthesized to determine the effects on activity of substitution at the 2 position of the flavanone ring. All were prepared by reacting the corresponding flavanone or chromanone with bis(dimethylamino)methane. In this way were prepared the 2-phenyl-, the 2-(4-pyridyl)-, and the 2-(2-thienyl)-3-methyleneflavanones **8–10** and the 2-substituted chromanones **11–14**.





8, aryl = phenyl
9, aryl = 4-pyridyl
10, aryl = 2-thienyl

11, R^1 = H; R^2 = 1-naphthyl
12, R^1 = H; R^2 = 2-naphthyl
13, R^1, R^2 = H
14, R^1, R^2 = CH_3

Discussion

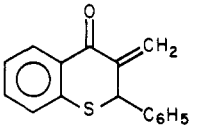
The 3-methyleneflavanones and their derivatives were tested in vitro for their ability to inhibit the growth of representative bacteria and fungi (See Table II for results). All the 3-methyleneflavanones displayed some degree of activity against Gram-positive bacteria and fungi. All were inactive when tested against the Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus vulgaris* at 100 mcg/mL, the highest concentration used in testing. The following points can be made concerning structure–activity relationships.

Both antifungal and antibacterial activity are increased when the phenyl ring is substituted with lipophilic electron-withdrawing groups. The 4′-chloro (**17**), the 2′,4′-dichloro (**18**), and the 4′-bromo analogues (**19**) are the most active flavanones. Activity is unaffected by the position of substitution on the phenyl ring. The 3′- and the 4′-chloro compounds **16** and **17** display similar activities against the test organisms. Electron donating substituents, such as dimethylamino in **21**, reduce activity below that of **2**. Hydrophilic substituents, such as the cyano in **24**, have activity similar to **2** but less activity than **17** or **18**.

In contrast, electron-withdrawing substituents on the benzo ring, as in **25** and **26**, lead to reduced activity when compared to the corresponding unsubstituted compounds **2** and **19**. However, electron-donating substituents in the benzo ring, as in **27** and **28**, appear to have little effect on either the profile or intensity of the antimicrobial response.

The presence of a phenyl ring at position 2 of the flavanone ring is not crucial to activity, since both the 1- and 2-naphthylchromanones **11** and **12** are approximately equal in activity to the phenyl compound **2**. Some type of aromatic ring is necessary, however, since 3-methylenechromanone (**13**) and 2,2-dimethyl-3-methylenechromanone (**14**) are largely inactive.

3-Methylene-1-thiaflavanone (**15**) was prepared by the



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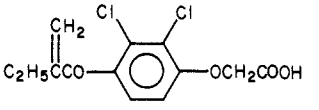
reaction of thiaflavanone with bis(dimethylamino)-methane. It did not inhibit the growth of any test organism, suggesting that an oxygen atom at position 1 is necessary for activity.

The introduction of a second aromatic ring at position 2 of the flavanone ring produced compounds **8–10**, having enhanced activities against Gram-positive bacteria but with reduced activity against other microorganisms. The 2-phenyl analogue **8** was further tested against an expanded spectrum of Gram-positive organisms, along with hexachlorophene and vioform,[4] two topical antimicrobial agents active against Gram-positive bacteria. As shown in Table III, **8** is roughly equal to hexachlorophene and is superior to vioform in inhibiting the growth of such microorganisms in vitro. However, unlike hexachlorophene, it displayed no activity against common fungi or Gram-negative bacteria.

It is possible that the chemical mechanism of action of the methyleneflavanones is a function of the reactivity of the α,β-unsaturated ketone moiety. For example, the diuretic ethacrynic acid **29**, which contains a similar α-



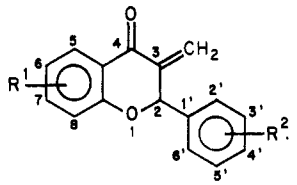
29

methylene ketone function, is thought to act by binding thiol groups by conjugate addition.[5] The methylene-

(3) E. C. Taylor and Y. Shvo, *J. Org. Chem.*, **33**, 1719 (1968).

(4) 5-Chloro-8-hydroxy-7-iodoquinoline: "Merck Index", 9th ed., Merck and Co., Rahway, NJ, 1976, p 664.

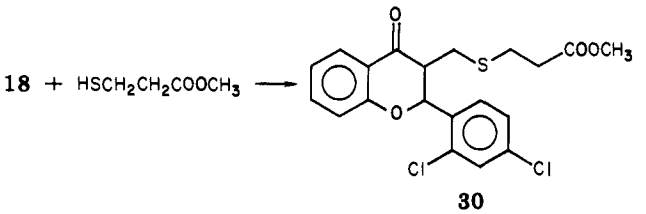
Table I. 3-Methyleneflavanones



no.	R^1	R^2	mp, °C	formula	method[a]	% yield	recrystn solvent	anal.
2	H	H	30-32	$C_{16}H_{12}O_2$	A	12	*b*	*c*
16	H	3'-Cl	85-87	$C_{16}H_{11}ClO_2$	A	5	*b*	*d*
17	H	4'-Cl	84-85	$C_{16}H_{11}ClO_2$	A	5	hexane	C, H
18	H	2',4'-Cl_2	86-87	$C_{16}H_{10}Cl_2O_2$	B	70	hexane	C, H
19	H	4'-Br	91-92	$C_{16}H_{11}BrO_2$	A	10	*b*	C, H
20	H	4'-OCH_3	*e*	$C_{17}H_{14}O_3$	A	5	*b*	*f*
21	H	4'-$N(CH_3)_2$	110-112	$C_{18}H_{17}NO_2$	A	11	$CHCl_3$-hexane	*g*
22	H	4'-CH_3	*e*	$C_{17}H_{14}O_2$	A	3	*b*	C, H
23	H	4'-$C(CH_3)_3$	109-110	$C_{20}H_{20}O_2$	B	6	*b*	*h*
24	H	4'-CN	*e*	$C_{17}H_{11}NO_2$	A	4	*b*	*i*
25	6-Br	H	*e*	$C_{16}H_{11}BrO_2$	B	34	*b*	*j*
26	6-Br	4'-Br	115-116	$C_{16}H_{10}Br_2O_2$	B	75	hexane	C, H
27	7-OCH_3	4'-Cl	97-99	$C_{17}H_{13}ClO_3$	B	42	*b*	*b*
28	6-CH_3	H	57-63	$C_{17}H_{14}O_2$	B	20	*b*	C, H

[a] Method A, prepared by decomposing the corresponding Mannich base. Method B, prepared by direct reaction of the flavanone with bis(dimethylamino)methane. [b] Purified by chromatography on silica gel. [c] Mass spectrum (70 eV) calcd for (M^+): *m/e* 236.0837. Found: 236.0884. [d] Mass spectrum (70 eV) calcd for (M^+): *m/e* 270.0447. Found: 270.0421. [e] Isolated as an oil. [f] Mass spectrum (70 eV) calcd for (M^+): *m/e* 266.0939. Found: 266.0916. [g] Mass spectrum (70 eV) calcd for (M^+): *m/e* 279.1255. Found: 279.1269. [h] Mass spectrum (70 eV) calcd for (M^+): *m/e* 292.1462. Found: 292.1553. [i] Mass spectrum (70 eV) calcd for (M^+): *m/e* 261.0787. Found: 261.0793. [j] Mass spectrum (70 eV) calcd for (M^+): *m/e* 313.9941, 315.9921. Found: 313.9962, 315.9852. [k] Mass spectrum (70 eV) calcd for (M^+): *m/e* 300.0552. Found: 300.0570.

flavanones exhibit a high degree of reactivity with thiols. One of the most active analogues, **18**, reacted rapidly and exothermically when treated with the model thiol methyl 3-mercaptopropionate to give the adduct **30**. Two inactive



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analogues (**4** and **7**) having substituents on the methylene function reacted very sluggishly or not at all with methyl 3-mercaptopropionate. These findings suggest the possibility that the antimicrobial activity of the methyleneflavalones is a consequence of their ability to covalently bind some nucleophilic receptor by conjugate addition to the α,β-unsaturated ketone system.

Experimental Section

All melting points are uncorrected. IR spectra were determined with a Perkin-Elmer Model 237 grating spectrophotometer; ^1H NMR spectra were taken on a Varian T-60 A spectrophotometer. High-resolution mass spectra were taken on an Associated Electronic Industries MS 902. Where analyses are indicated by symbols, the values were within ±0.4% of the calculated values.

All compounds were homogeneous on TLC using silica gel plates (E. Merck and Co.) eluting with $CHCl_3$–EtOAc mixtures.

Flavanones. When not commercially available, the flavanone and chromanone starting materials were prepared by methods similar to those described in the literature. Five of the flavanones so prepared were new compounds and were purified and characterized (see Table IV).

3-Methyleneflavanone (2). Method A. A solution of 10 g of 3-[(dimethylamino)methyl]flavanone hydrochloride in 400 mL of H_2O was heated on the steam bath for 2 h. When cool, the cloudy solution was extracted with ether. The ether phase was dried, filtered, and evaporated. This gave 2.2 g of an oil, which was taken up in hot pentane, filtered, and concentrated to 10 mL. After cooling to room temperature, the solution was decanted from a small amount of gummy precipitate and evaporated to give 300 mg of **2** as a low-melting, waxy white solid.

2',4'-Dichloro-3-methyleneflavanone (18). Method B. A mixture of 3 g (0.01 mol) of 2',4'-dichloroflavanone and 27 mL of bis(dimethylamino)methane was stirred at room temperature while 27 mL of acetic anhydride was added dropwise over 15 min. A slight temperature rise occurred. One hour later the reaction was partitioned between 200 mL of ether and 200 mL of cold H_2O. The organic phase was separated, washed with saturated aqueous $NaHCO_3$, filtered, and evaporated. The residue was chromatographed on silica gel eluting with benzene. This gave 2.5 g of flavanone **18** as white crystals, mp 86–87 °C, after recrystallization from hexane.

3-[(*N*,*N*-Dimethylamino)methyl]-3-methylflavanone (3) Hydrochloride. A solution of 5 g (21 mmol) of 3-methylflavanone, 3.42 g (42 mmol) of dimethylamine hydrochloride, 1.3 g (42 mmol) of paraformaldehyde, 6 drops of concentrated HCl, and 50 mL of 2-propanol was refluxed for 1 h. When cool, solvent was removed and the residue was partitioned between ether and 5% HCl. The ether phase was washed twice with 5% HCl, and the combined aqueous extracts were made basic by the addition of solid K_2CO_3. The amine was extracted into ether, which was dried and evaporated to give a yellow oil. It was taken up in ethyl acetate and treated with HCl to give 100 mg of the HCl salt of **3** as a white solid, mp 163–164 °C. Anal. ($C_{19}H_{22}ClNO_2$) C, H, N.

3-[(Dimethylamino)methylene]flavanone (4). A mixture of 2.2 g of flavanone and 6 g of dimethylformamide dimethyl acetal was refluxed for 7 days. The volatile products were removed under reduced pressure, and the residue was recrystallized from benzene–hexane to give 500 mg of **4** as yellow crystals: mp 167 °C; IR ($CHCl_3$) 1650 cm^{-1} (CO). Anal. ($C_{17}H_{16}NO_2$) C, H, N.

3-(Hydroxymethylene)flavanone (5). A mixture of 1.7 g of 3-[(dimethylamino)methylene]flavanone (**4**), 4 mL of concentrated

(5) D. A. Koechel and G. O. Rankin, *J. Med. Chem.*, 21, 764 (1978).

Table II. Antimicrobial Activity of 3-Methylenechromanones and Flavanones[a]

no.	*Staphylococcus aureus*[b]	*Streptococcus faecalis*[c]	*Candida* species[d]	Dermatophyte fungi[e]	*Aspergillus niger*[f]
1	6.2-12.5	50	12.5-100	≤3.1-12.5	50
2	6.2-12.5	100	50-100	6.2-12.5	100
3	>50	>50	>50	>50	>50
4	>100	>100	>100	100	>100
7	>100	>100	>100	>100	>100
8	0.25-0.5	0.78	>100	>100	>100
9	0.78	3.1	100 to >100	12.5-25	>100
10	0.5	1.0	>100	6.2-25	>100
11	≤3.1-6.2	50	50 to >100	6.2-12.5	>100
12	≤3.1-6.2	50	25 to >100	6.2-25	>100
13	50	100	25-50	6.2-12.5	100
14	>100	>100	50-100	25-50	>100
15	50-100	>100	>100	50 to >100	>100
16	≤3.1-6.2	25	25-50	≤3.1-12.5	100
17	≤3.1	25	12.5-25	≤3.1-25	25
18	1.56	25-50	25-50	6.2-25	100
19	≤3.1-6.2	25	25	6.1-12.5	50
20	12.5	>100	50 to >100	12.5-25	100
21	50	>100	100 to >100	12.5-25	100
22	6.2	50	25-50	≤3.1-6.2	50
23	≤3.1-6.2	25-50	50 to >100	12.5-25	100
24	12.5	100	50-100	6.2-25	50
25	50	>100	>100	100 to >100	>100
26	6.2-12.5	>100	100 to >100	12.5-50	>100
27	≤3.1-6.2	50	25 to >100	6.2-12.5	25
28	6.2	50	25	6.2	25
29	>100	>100	>100	>100	>100

[a] Minimum inhibitory concentration of drug in mcg/mL. [b] ATCC 6538, NRRL B2747. [c] ATCC 10541. [d] *C. albicans* ATCC 10231, NRRL Y-477; *C. krusei* VM 29B; *C. tropicalis* NRRL Y1410; *C. quilliermondii* VM 42. [e] *Trichophyton mentagrophytes* ATCC 4807, TM-2, CDC; *T. rubrum* ATCC 10218; *Microsporum gypseum* ATCC 14683. [f] ATCC 16404.

Table III. Antimicrobial Activity[a] of 3-Methylene-2-phenylflavanone (8)

organism	8	hexachlorophene	vioform[b]
Staph. aureus ATCC 6538	0.5	0.5	12.5
Staph. aureus ATCC 151	0.25	0.5	3.1
Staph. aureus NRRL B2747	0.5	0.5	12.5
Staph. aureus 9-267	0.5	1.0	12.5
Staph. aureus 9-195	0.5	1.0	12.5
Staph. epidermidis ATCC 12228	0.25	0.5	12.5
Strep. faecalis ATCC 10541	1.0	0.125	12.5
Enterococcus 9-25	1.0	1.0	25
Sarcina subflava ATCC 7468	1.0	0.06	3.1
Bacillus subtilis ATCC 6633	0.25	0.125	6.2
Clostridium acetobutylicum ATCC 824[c]	1.0	0.06	≤6.2
Propionobacterium acnes ATCC 6919[c]	3.1	0.06	25

[a] Activity expressed as minimum inhibitor concentration in mcg/mL. [b] Reference 4. [c] Anaerobes incubated for 72 h at 37 °C.

HCl, and 100 mL of 2-propanol was heated to reflux and then allowed to cool to room temperature and stand for 1 day. Evaporation gave a residue, which was extracted with boiling ether. The ether was filtered, concentrated to a 10-mL volume, and diluted with 100 mL of hexane. A solid crystallized that amounted to 500 mg of **5**: mp 116 °C; IR ($CHCl_3$) 1630 cm^{-1} (CO); MS Calcd for $C_{16}H_{12}O_3$, *m/e* 252.07830. Found, 252.07872 (M^+).

3-(Chloromethylene)flavanone (7). A solution of 1.44 g (6 mmol) of 3-(hydroxymethylene)flavanone (**5**) and 640 mg of triethylamine in 25 mL of THF was cooled to 0 °C while stirring under an inert atmosphere. To this was added dropwise 730 mg (6 mmol) of methanesulfonyl chloride in a small volume of THF. The reaction was allowed to stir at room temperature for 1 day and filtered, and the filtrate was evaporated. The residue was taken up in ether, washed with H_2O, saturated aqueous $NaHCO_3$ solution, 1% HCl, and H_2O, and dried over anhydrous Na_2SO_4. Filtration and evaporation gave 2.2 g of 3-[(methanesulfonyl)methylene]flavanone (**6**) as an oil.

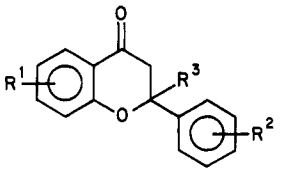
This oil was dissolved in 50 mL of acetonitrile and combined with 1.8 g of powdered NaCl, 0.2 g of 18-crown-6, and 5 drops of concentrated H_2SO_4. After stirring at room temperature for 2 weeks, the reaction mixture was filtered and concentrated under reduced pressure. The residue was extracted with benzene and the benzene evaporated to give 2 g of a tan oil. The oil was chromatographed on 200 g of silicic acid eluting with benzene to give 940 mg (60% yield) of **7** as a clear, pale yellow oil: IR (CCl_4) 1680 cm^{-1} (CO); ^1H NMR (CCl_4) δ 6.5 (m, 1 H); MS Calcd for $C_{16}H_{11}ClO_2$: *m/e* 270.04469. Found: 270.04277 (M^+).

3-Methylene-2-phenylflavanone (8). A solution of 2-phenylflavanone in 12 mL of bis(dimethylamino)methane was stirred under argon while 12 mL of acetic anhydride was added dropwise. After 1 h, the reaction was poured over ice and partitioned into an equal volume of ether. The ether phase was dried and evaporated to give a solid, which was recrystallized from hexane to give 930 mg (22% yield) of **8** as white crystals: mp 165–167 °C; IR ($CHCl_3$) 1675 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 5.03, 6.65 (m, 2 H, CH_2). Anal. ($C_{22}H_{16}O_2$) C, H.

3-Methylene-2-(4-pyridyl)flavanone (9). To a solution of 890 mg of 2-(4-pyridyl)flavanone in 20 mL of bis(dimethylamino)methane was added 20 mL of acetic anhydride over a 20-min period. After 1 h, the reaction was poured over ice and the product was collected by filtration. After drying, this amounted to 500 mg (55% yield) of **9** as a white solid, mp 159–160 °C; IR ($CHCl_3$) 1675 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 5.05, 6.62 (m, 2 H, CH_2). Anal. ($C_{21}H_{15}NO_2$) C, H, N.

3-Methylene-2-(2-thienyl)flavanone (10). A solution of 1.5 g of 2-(2-thienyl)flavanone in 15 mL of bis(dimethylamino)methane was stirred at room temperature while 15 mL of acetic anhydride was added dropwise over a 15-min period. After stirring overnight the reaction was poured over ice. The product precipitated as a tan solid, which was collected by filtration and

Table IV. Substituted Flavanones



R^1	R^2	R^3	mp, °C	formula	% yield[a]	recrystn solvent	anal.
H	2',4'-Cl_2	H	92	$C_{15}H_{10}Cl_2O_2$	26	MeOH	C, H
H	4'-$C(CH_3)_3$	H	104	$C_{19}H_{20}O_2$	10	hexane	C, H
6-Br	4'-Br	H	168	$C_{15}H_{10}Br_2O_2$	13	ether	C, H
7-OCH_3	4'-Cl	H	122	$C_{16}H_{13}ClO_3$	10	[b]	C, H
H	H	2-thienyl	80	$C_{19}H_{14}SO_2$	2	[b]	H; C[c]

[a] Yield calculated from the starting acetophenone. [b] Purified by chromatography on silica gel. [c] C: calcd, 74.48; found, 75.39.

air-dried to give 1.1 g (70% yield) of the methyleneflavanone **10**: IR ($CHCl_3$) 1680 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 5.17, 6.58 (m, 2 H, CH_2). Anal. ($C_{20}H_{14}SO_2$) C, H.

3-Methylene-2-(1-naphthyl)chromanone (11). A mixture of 33.4 g (0.121 mol) of 2-(1-naphthyl)chromanone, 19.7 g (0.242 mol) of dimethylamine hydrochloride, 7.26 g (0.242 mol) of paraformaldehyde, 1 mL of concentrated HCl, and 100 mL of 2-propanol was refluxed for 1 h. It was cooled and concentrated, and the residue was partitioned between 400 mL of ether and 1 L of 5% aqueous HCl. The aqueous phase was separated, washed with 200 mL of ether, cooled to 5 °C, and made basic with solid K_2CO_3. The liberated base was extracted into ether, which was dried and concentrated under reduced pressure. The residue was taken up in 100 mL of ethyl acetate and treated with acetyl chloride/ethanol. On cooling, white crystals precipitated. Recrystallization from ethanol–ethyl acetate gave 9.8 g (22% yield) of the HCl salt of 3-[(dimethylamino)methyl]-2-(1-naphthyl)-chromanone as white crystals, mp 176–178 °C. Anal. Calcd for $C_{22}H_{22}ClNO_2$: C, 71.82; H, 6.03; N, 3.81. Found: C, 71.96; H, 6.23; N, 3.88.

A solution of 2 g of the Mannich base in 450 mL of H_2O was stirred at room temperature for 3 days and then extracted with ethyl acetate. The ethyl acetate was dried over anhydrous $MgSO_4$, filtered, and evaporated to give 1 g (48% yield) of **11**, mp 140–141 °C. MS Calcd for $C_{20}H_{14}O_2$: *m/e* 286.09900. Found: 286.10210 (M^+).

3-Methylene-2-(2-naphthyl)chromanone (12). Using the procedure described for **11**, 31 g (0.113 mol) of 2-(2-naphthyl)-chromanone was converted to 4.1 g (10% yield) of the HCl salt of the Mannich base 3-[(dimethylamino)methyl]-2-(2-naphthyl)chromanone, isolated as a white solid, mp 125–145 °C after recrystallization from acetone. Anal. Calcd for $C_{22}H_{22}ClNO_2$: C, 71.82; H, 6.03; N, 3.81. Found: C, 70.90; H, 6.15; N, 3.61.

The filtrate from the crystallization of the HCl salt was evaporated to give 4 g of a semisolid residue. Chromatography on silica gel eluting with benzene gave 1.15 g (3% yield) of **12** as a solid, mp 95–98 °C. MS. Calcd for $C_{20}H_{14}O_2$: *m/e* 286.09900. Found: 286.09890 (M^+).

3-Methylenechromanone (13). A solution of 5 g (0.034 mol) of 4-chromanone in 50 mL of bis(dimethylamino)methane was stirred at room temperature while 50 mL of acetic anhydride was added dropwise over a 20-min period. After 3 h the reaction was poured over ice and extracted into ether. Evaporation gave a yellow oil, which was chromatographed on 200 g of silica gel eluting with benzene. This gave 940 mg (17% yield) of **13** as a low-melting yellow solid: IR ($CHCl_3$) 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.97 (m, 2 H, ring methylene), 5.55, 6.23 (m, 2 H, vinyl methylene). MS Calcd for $C_{10}H_6O_2$: *m/e* 160.05238. Found: 160.05128.

2,2-Dimethyl-3-methylenechromanone (14). Three grams (0.017 mol) of 2,2-dimethylchromanone was converted to the 3-methylene derivative as above. Chromatography on 125 g of silica gel eluting with 95:5 (v/v) hexane/MeOH gave 250 mg (8% yield) of **14** as a yellow oil: IR ($CHCl_3$) 1680 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 5.26, 6.42 (m, 2 H, vinyl methylenes). MS Calcd for $C_{12}H_{12}O_2$: *m/e* 188.0837. Found: 188.0872.

3-Methylene-1-thiaflavanone (15). Three grams (0.0125 mol) of 1-thiaflavanone was converted to the 3-methylene derivative as above. Chromatography on 200 g of silica gel eluting with benzene gave 1.62 g (51% yield) of **15** as a pale yellow oil: IR ($CHCl_3$) 1665 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 5.25 (m, 1 H, 5.25, 6.25 (m, 2 H, vinyl methylenes). MS Calcd for $C_{16}H_{12}SO$: *m/e* 252.06082. Found: 252.05946 (M^+).

3-[[(2-Carbomethoxyethyl)thio]methyl]-2',4'-dichloroflavanone (30). To a solution of 2.0 g (7 mmol) of **18** in 50 mL of dry ether was added 830 mg (7 mmol) of methyl 3-mercaptopropionate. A slight exotherm occurred and the TLC indicated that complete reaction had occurred. The solution was evaporated and the residue was purified by chromatography on silica gel eluting with toluene to give 800 mg (27% yield) of **30** as a pale yellow oil: IR ($CHCl_3$) 1750 (ester CO), 1700 cm^{-1} (ketone CO); ^1H NMR ($CDCl_3$) δ 2.7 (m, 6 H, $CH_2SCH_2CH_2$), 3.3 (m, 1 H, ArCOCH), 3.6 (s, 3 H, OCH_3), 6.0 (d, 1 H, J = 12 Hz, ArCH), 7.0–8.2 (m, 7 H, aromatic). MS Calcd for $C_{20}H_{18}Cl_2SO_4$: *m/e* 424.03017. Found: 424.02764 (M^+).

Antimicrobial Activity. Compounds were tested for in vitro antimicrobial activity using an agar dilution method. For each compound, stock solutions and twofold serial dilutions were prepared in either *N,N*-dimethylformamide (DMF) or 50% aqueous DMF. Aliquots of each dilution were added to liquid Eugon agar (Difco) in a ratio of 1 part test solution to 100 parts agar medium to yield final test concentrations in agar medium of 100, 50, 25, 12.5, 6.2, and 3.1 mcg/mL and a noninhibitory residual DMF level of ≤1%. In some cases, tests with further dilutions were conducted. After hardening and drying, the agar surface was inoculated with standardized suspensions of test organisms using a Steer's multiple-inocula replicating device. For preparation of inocula, aerobic organisms were cultured on Eugon agar (Difco) slants at 32 °C, bacteria and yeasts for 24 h and filamentous fungi for 1 week. Inocula were prepared by suspending cells or spores from a slant culture in sufficient sterile diluent, either Eugon broth (Difco) or saline, to yield cell or spore concentrations of 10^6/mL. Anaerobic bacteria were cultured in fluid thioglycollate medium (Difco) for 72 h at 32 °C and used undiluted as inocula. Minimum inhibitory concentrations (MIC) representing the lowest concentration of test compound which inhibited the growth of test organisms were determined after 24 h at 32 °C for bacteria and yeasts, after 48–72 h at 32 °C for filamentous fungi, and after 72 h anaerobic incubation at 37 °C (Gas-Pak anaerobic jar, BBL) for anaerobic bacteria.