ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1986, p. 375–381
0066-4804/86/090375-07$02.00/0

Vol. 30, No. 3

Synthesis and Antimicrobial Activity of a Series of Caespitin Derivatives

CORNELIS J. VAN DER SCHYF,†* THEODOR G. DEKKER, THEUNIS G. FOURIE, AND FRIEDRICH O. SNYCKERS

Central Research Laboratory, Noristan Limited, Silverton 0127, Republic of South Africa

Received 19 December 1985/Accepted 28 April 1986

Chemical modification of the naturally occurring phlorophenone antimicrobial agent caespitin is described. These modifications include variations in the phenone side chain, substitution with prenyl, allyl, and benzyl in the 4-position of the phlorophenone nucleus, and ring cyclizations via etherification to give furan and chroman compounds. Several of these derivatives show enhanced in vitro potency over caespitin. Studies on the development of microbial resistance against these compounds show that no or very little resistance developed after several passes of these compounds in representative microbial strains.

Caespitin [C-1; 2-(4-methylpentanoyl)-4-(3-methylbuten-2-yl)phloroglucinol] is a naturally occurring phlorophenone derivative isolated from the indigenous southern African plant *Helichrysum caespititium* (2). The antimicrobial activity of phlorophenones and related compounds isolated as natural products or synthesized de novo has been reported as early as 1954 (8) and investigated since (6, 7). The in vitro potency of caespitin and the compounds studied previously (6–8) against a variety of bacteria and fungi has not been found comparable to that of most of the antimicrobial agents currently in use. Comprehensive studies done on the antimicrobial and pharmacological action of these compounds, including studies on the development of microbial resistance, are still lacking. Consequently, a research program was initiated to investigate these aspects in an endeavor to optimize the antimicrobial potency and spectrum of phloroglucinol-derived compounds. A synthesis program led to the production of 42 compounds related to caespitin (1), which were tested in vitro against a selection of gram-positive and gram-negative bacteria, yeasts, and fungi.

MATERIALS AND METHODS

Microorganisms. The bacteria used were obtained from the National Collection of Type Cultures, London, England, and the American Type Culture Collection, Rockville, Md., as freeze-dried cultures. The strains used were *Streptococcus pyogenes* NCTC 8198, *Proteus mirabilis* NCTC 8559, *Staphylococcus aureus* ATCC 6538, *S. aureus* NCTC 6571A, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027. Upon receipt, the strains were cultured onto tryptone soy agar (TSA; Oxoid CM131) with the addition of 10% horse blood for *S. pyogenes* and maintained at refrigerator temperature until used. Yeasts and fungi were obtained from the American Type Culture Collection and from the National Collection of Pathogenic Fungi, London, England, and included the following: *Candida albicans* ATCC 10231, *Candida tropicalis* NCPF 3111, *Absidia corymbifera* NCPF 2001, *Aspergillus fumigatus* NCPF 2140, *Sporotrichum schenkii* NCPF 3182, *Trichophyton rubrum* NCPF 197, *T. mentagrophytes* NCPF 410, and *Microsporum canis* NCPF 351. The organisms were maintained on Sabouraud dextrose agar (SDA; Oxoid CM41) until used.

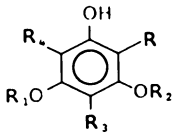
MIC determinations. The MIC was determined by a tube dilution method. Standard inocula for the tests on bacteria were prepared by incubating slopes of TSA inoculated with the organisms for 24 h at 37°C, from which suspensions yielding ca. 10^6 CFU/ml were obtained for use in the experiments. Twofold serial dilutions of each test compound were prepared in brain-heart infusion (BHI; Oxoid CM225) broth down to 1 μg/ml. A growth control of BHI broth was included in each series. To each of the prepared sets of concentrations of each test compound was added 0.1-ml portions of the organism suspensions. The MIC was defined as the lowest concentration of a compound preventing visual growth after 48 h at 37°C. Suspensions of the two yeasts, *C. albicans* and *C. tropicalis*, obtained from SDA cultures were diluted in sterile physiological saline to give 10^6 CFU/ml. Cultures of the molds in Sabouraud liquid medium (SLM; Oxoid CM146) were kept at 4°C until used and then incubated at 37°C for 5 to 14 days. For use, the suspensions were diluted to contain ca. 10^6 CFU/ml. Concentrations of each of the compounds were prepared in SLM in twofold steps from 100 to 0.1 μg/ml. The same procedure for testing against the bacteria was used for the fungi, only with SLM. Growth controls of SLM were included in each series and acted as indicators to determine the time (5 to 15 days, 37°C) at which the test broths were examined for evidence of growth. The MIC was similarly indicated by the lowest concentration of test compound to prevent growth.

MBC determinations. Subcultures were made from all the broths showing no growth as well as from the growth controls. The subcultures were made onto BHI agar (Oxoid CM375) for the bacteria and onto SDA for the fungi. The plates were incubated at 37°C for 48 h for bacteria and 5 to 15 days for fungi. After incubation the plates were examined for growth, and the lowest concentration allowing no evident growth was recorded as the MBC.

Screening for antimicrobial resistance. The microorganisms used were *S. aureus* NCTC 6571A, *S. pyogenes* NCTC 8198, and *C. albicans* ATCC 10231, from which standard inocula containing 10^6 CFU/ml were obtained. Six compounds were tested in two series of three compounds each, the first series including compounds C-1, C-31, and C-33 and the second series compounds C-13, C-24, and C-39.

* Corresponding author.
† Present address: Department of Pharmaceutical Chemistry, Potchefstroom University for Christian Higher Education, Potchefstroom 2520, South Africa.

A

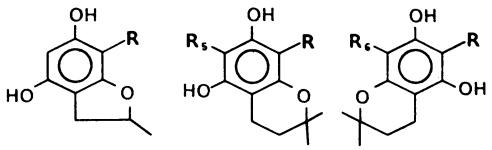


Compound	R	R_1	R_2	R_3	R_4
C-1	$-C{:}OCH_2CH_2CH(CH_3)_2$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-2	-H	H	H	H	H
C-3	$-C{:}OCH_3$	H	CH_3	H	H
C-4	$-C{:}OCH_3$	CH_3	CH_3	H	H
C-5	$-C{:}OCH_2CH_2CH_3$	H	H	H	H
C-6	-H	H	H	$-CH_2CH{:}CH_2$	H
C-7	$-C{:}OCH_2CH_3$	H	H	$-CH_2CH{:}CH_2$	H
C-8	$-C{:}OCH_2CH_2CH_3$	H	H	$-CH_2CH{:}CH_2$	H
C-9	$-C{:}OCH_2CH(CH_3)_2$	H	H	$-CH_2CH{:}CH_2$	H
C-10	$-C{:}O(CH_2)_3CH_3$	H	H	$-CH_2CH{:}CH_2$	H
C-11	$-C{:}OCH_2CH_2OCH_2CH_3$	H	H	$-CH_2CH{:}CH_2$	H
C-12	$-C{:}OCH_2CH_2CH(CH_3)_2$	H	H	$-CH_2CH{:}CH_2$	H
C-13	$-C{:}O(CH_2)_4CH_3$	H	H	$-CH_2CH{:}CH_2$	H
C-14	$-C{:}O\overline{CH(CH_2)_4CH_2}$	H	H	$-CH_2CH{:}CH_2$	H
C-15	$-C{:}OCH_2C_6H_4Cl(p)$	H	H	$-CH_2CH{:}CH_2$	H
C-16	-H	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-17	$-C{:}OCH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-18	$-C{:}OCH_2CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-19	$-C{:}OCH_2CH_2CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-20	$-C{:}O(CH_2)_3CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-21	$-C{;}OCH_2CH(CH_3)_2$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-22	$-C{:}O\overline{CH(CH_2)_3CH_2}$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-23	$-C{:}O(CH_2)_4CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-24	$-C{:}O\overline{CH(CH_2)_4CH_2}$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-25	$-C{:}O(CH_2)_5CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-26	$-C{:}OCH_2C_6H_4Cl(p)$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-27	$-C{:}OCH_2C_6H_5$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-28	$-C{:}O(CH_2)_6CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-29	$-C{:}O(CH_2)_7CH_3$	H	H	$-CH_2CH{:}C(CH_3)_2$	H
C-30	$-C{:}OCH_2CH_2CH_3$	H	H	$-CH_2C_6H_5$	H
C-31	$-C{:}OCH_2CH_2CH(CH_3)_2$	H	H	$-CH_2C_6H_5$	H
C-32	$-C{:}O(CH_2)_7CH_3$	H	H	$-CH_2C_6H_5$	H
C-33	$-C{:}OCH_2CH_2CH_3$	H	H	$-C{:}OCH_2CH_2CH_3$	H
C-42	$-C{:}OCH_2CH_2CH_3$	H	H	$-CH_2CH{:}CH_2$	$-C{:}OCH_2CH_2CH_3$

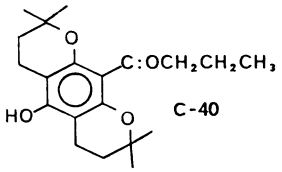
FIG. 1. (A) Structural features of caespitin and analogs. (B) Structural features of furan and chroman analogs.

These compounds were initially tested in duplicate against the bacteria and yeasts to determine their prepassage MICs. Since Iso-Sensitest broth (ISB; Oxoid CM473) is a highly suitable medium for resistance studies on bacteria, this was used to prepare concentrations of each of the first-series compounds in twofold steps from 1,000 to 1 μg/ml. Due to discrepancies (see Results) in MICs obtained with ISB in this test and the previously performed MIC determinations done in BHI broth, resistance studies on the second-series compounds were done in the latter medium. For the initial MIC determinations against *C. albicans*, concentrations of each of the compounds were prepared in SLM in twofold steps from 100 to 0.1 μg/ml. As reference compounds, streptomycin (potency 735 U/mg, lot no. 21F-0399; Sigma Chemicals, London), nitrofurantoin (lot no. 29C-0179; Sigma Chemicals), and nystatin (5,600 U activity per mg, lot no. 120F-0638; Sigma Chemicals) were used. Twofold series were prepared from stock solutions containing 1,000 μg/ml for streptomycin and 10,000 μg/ml for nitrofurantoin and nystatin. A growth control of medium only was included in each series. Inoculation, incubation, and determination of the MICs proceeded as described above. The MICs were confirmed by repeat testing, and the mean value obtained was used to calculate the initial subinhibitory concentrations of the test and reference compounds for use in the passages. To obtain a passage series, the three test organisms were

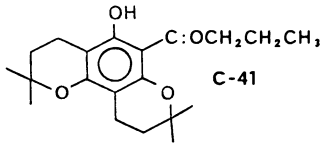
B



Compound	R	R_5	R_6
C-34	-C:OCH$_2$CH$_2$CH$_3$	-	-
C-35	-C:OCH$_2$CH$_2$CH$_3$	H	-
C-36	-C:OCH$_2$CH$_2$CH(CH$_3$)$_2$	-	H
C-37	-C:OCH$_2$CH$_2$CH(CH$_3$)$_2$	H	-
C-38	-C:O(CH$_2$)$_7$CH$_3$	-	H
C-39	-C:O(CH$_2$)$_7$CH$_3$	H	-
C-43	-C:OCH$_2$CH$_2$CH$_3$	-C:OCH$_2$CH$_2$CH$_3$	-



C-40



C-41

sequentially subcultured into liquid media containing 25, 50, 100, 200, 400%, etc., of the MICs of the six compounds until a terminal concentration was reached at which growth did not take place. The concentrations and the number of passages required to reach a resistant state or terminal concentration were noted.

Cross-resistance determinations. The passaged organisms from the resistance experiments were maintained on agar medium containing a subinhibitory concentration of the relevant compound and used when applicable (i.e., when increased resistance developed) for cross-resistance determinations against a range of commonly used antimicrobial agents. The MICs were determined by using APO4 Sensititre plates (Seward Laboratories, London), following procedure recommended by the manufacturer. These plates contained the antimicrobial agents listed in Table 7. The resistant passaged strains were tested to compare them with their sensitive nonpassaged parent strains. Any significant increase in the MIC for the passaged strains was taken as evidence of induced cross-resistance.

General chemistry methods. Methods and physical data for all compounds listed as active in Tables 1 and 2 are reported in this section. Melting points (mp) were determined with a Reichert hot-stage apparatus and are uncorrected, Infrared (IR) spectra were obtained with a Unicam SP1050 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WP-80 spectrometer with tetramethylsilane (TMS) as the internal reference. Mass spectra (MS) were determined with an AEI MS-12 spectrometer. Preparative chromatography was carried out on a Waters/LC System 500, and Silica Gel 60 (70–230 mesh; Merck & Co., Inc., Rahway, N.J.) was used for column chromatography. Elemental analyses were performed on a Perkin-Elmer 240 analyzer. Physical data of compounds previously reported by us (1–3) are referred to by the appropriate literature references. ^{13}C NMR multiplicities were obtained from off-resonance proton decoupling, and assignments can be made according to the criteria set forth in reference 3. Elemental analyses and molecular ions of all compounds were in accordance with calculated values (C, ±0,4%; H, ±0,2%; m/z = M^+).

General procedure for the preparation of allyl-, prenyl-, and benzylphlorophenones (C-1 and C-6 through C-32). The general method previously reported by us (1), in which the appropriate phlorophenone is treated with either allyl-, prenyl-, or benzylchloride in an alkaline two-phase (aqueous-ether) system and catalyzed by CuCl, was used. The compounds produced, the analytical method used, and the appropriate references are as follows. 2-(4-Methylpentanoyl)-4-(3-methylbuten-2-yl)phloroglucinol (C-1; caespitin) (1–3); 2-propanoyl-4-(propen-2-yl)phloroglucinol (C-7) (1, 3); 2-butyryl-4-(propen-2-yl)phloroglucinol (C-8), mp 144 to 145°C, ^{13}C NMR (3); 2-(3-methylbutyryl)-4-(propen-2-yl)phloroglucinol (C-9), mp 119 to 121°C, ^{13}C NMR (3); 2-pentanoyl-4-(propen-2-yl)phloroglucinol (C-10), mp 123 to 126°C, ^{13}C NMR (3); 2-(4-methylpentanoyl)-4-(propen-2-yl)phloroglucinol (C-12), mp 140 to 143°C, ^{13}C NMR (3); 2-hexanoyl-4-(propen-2-yl)phloroglucinol (C-13), mp 118 to 120°C, ^{13}C NMR (3); 2-hexahydrobenzoyl-4-(propen-2-yl)phloroglucinol (C-14), mp 160 to 162°C, ^{13}C NMR (3); 2-(4-chlorophenyl)acetyl-4-(propen-2-yl)phloroglucinol (C-15), mp 181 to 183°C, ^{13}C NMR (3); 2-acetyl-4-(3-methylbuten-2-yl)phloroglucinol (C-17), mp 174 to 178°C, ^{13}C NMR (3); 2-propanoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-18), mp 156 to 157°C, ^{13}C NMR (3); 2-butyryl-4-(3-methylbuten-2-yl)phloroglucinol (C-19) (1, 3); 2-pentanoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-20), mp 126 to 127°C, ^{13}C NMR (3); 2-(3-methylbutyryl)-4-(3-methylbuten-2-yl)phloroglucinol (C-21), mp 116 to 119°C, ^{13}C NMR (3); 2-(2-cyclopentyl-1-oxo)-4-(3-methylbuten-2-yl)phloroglucinol (C-22), mp 128 to 130°C, mass spectrum m/z 290 (M^+), 235, 221, 207, 191, 165 (100%), 153, 139, 97, and 69; 2-hexanoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-23), mp 122 to 123°C, ^{13}C NMR (3); 2-hexahydrobenzoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-24) (1, 3); 2-heptanoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-25), mp 136 to 138°C, ^{13}C NMR (3); 2-(4-chlorophenyl)acetyl-4-(3-methylbuten-2-yl)phloroglucinol (C-26) (1, 3); 2-phenylacetyl-4-(-3-methylbuten-2-yl)phloroglucinol (C-27), mp 156 to 164°C, ^{13}C NMR (δ, $CDCl_3$ + CD_3OD), 203.3(s), 163.2(s), 162.1(s), 160.3(s), 136.1(d), 132.8(s), 129.9(d, 2C), 128.3(d, 2C), 126.5(s), 122.7(d), 106.8(s), 104.8(s), 94.5(d), 49.9(t), 25.7(q), 21.5(t), and 17.8(q); 2-octanoyl-4-(3-methylbuten-2-yl)phloroglucinol (C-28), mp 140 to 142°C, ^{13}C NMR (3); 2-butyryl-4-benzylphloroglucinol (C-30), mp 135 to 136°C, ^{13}C NMR (3); 2-(4-methylpentanoyl)-4-benzylphloroglucinol (C-31), mp 121 to 122°C, ^{13}C NMR (3); 2-nonanoyl-4-benzylphloroglucinol (C-32), (1, 3).

Butyrylphloroglucinol (C-5) was prepared by treating phloroglucinol with butyronitrile and zinc chloride-HCl according to the method described by Hoesch (5); mp 179 to 180°C, ^{13}C NMR (δ, DMSO-d_6), 205.1(s), 164.5(s), 164.2(s, 2C), 103.9(s), 94.7(d, 2C), 45.1(t), 17.8(t), and 13.8(q).

2,4-Dibutyrylphloroglucinol (C-33): To a solution of butyryl phloroglucinol (C-5; 4.6 g, 0.025 mol) and aluminum chloride (15 g) in carbon disulfide (20 ml), nitrobenzene (15 ml) was added over a period of 30 min under heavy stirring.

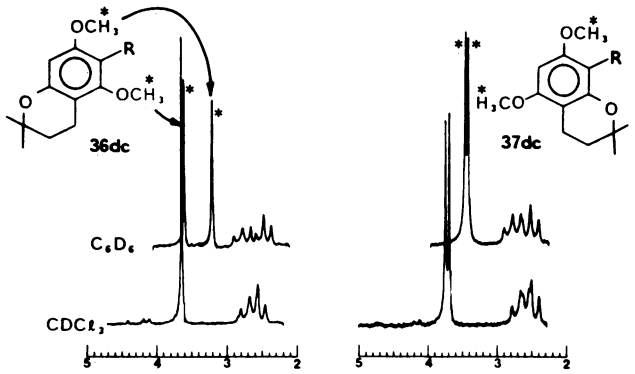


FIG. 2. ^{1}H NMR spectra (2 to 5 ppm) of the dimethoxychromans 36dc and 37dc, derived from C-36 and C-37, respectively. The upper tracings were done in C_6D_6 and the lower ones in $CDCl_3$. TMS was taken as the internal reference (0.00 ppm).

The reaction mixture was heated to 46°C, after which a mixture of butyrylchloride (8 ml) and nitrobenzene (5 ml) was added dropwise over a period of 30 min, refluxed for a further 2 h, and poured into ice-cold water (500 ml) containing 20 ml of concentrated HCl. Nitrobenzene was removed by steam distillation, and the mixture was allowed to stand overnight. The crystals were filtered, washed with petroleum ether, and chromatographed to obtain pure 2,4-dibutyrylphloroglucinol (C-33), mp 125 to 126°C, ^{13}C NMR (δ, DMSO-d_6), 205.9(s, 2C), 170.9(s), 168.2(s, 2C), 103.5(s, 2C), 94.8(d), 45.3(t, 2C), 17.5(t, 2C), and 13.7(q, 2C).

7-Butyryl-4,6-dihydroxy-2-methyl-2,3-dihydrobenzofuran (C-34): Aluminum chloride (48 g) and 2-(propen-2-yl)phloroglucinol (C-6, 12 g) were dissolved in 60 ml of carbon disulfide. To this solution nitrobenzene (45 ml) was added under heavy stirring over a period of 30 min. A solution of butyryl chloride (12.7 g) in 5 ml of nitrobenzene was then added over a period of 20 min to the latter mixture, after which refluxing proceeded for another 4 h. The mixture was cooled to room temperature and finally poured into ice-cold diluted HCl. The latter mixture was extracted with ether, dried over sodium sulfate, chromatographed, and recrystallized from benzene; mp 121 to 123°C, ^{13}C NMR (δ, DMSO-d_6), 203.6(s), 164.5(s), 162.5(s), 160.6(s), 103.9(s), 100.7(s), 95.4(d), 81.7(d), 43.8(t), 32.8(t), 21.6(q), 17.7(t), and 13.7(q).

5,7-Dihydroxy-2,2-dimethyl-6-(4-methylpentanoyl)chroman (C-36) and 5,7-dihydroxy-2,2-dimethyl-8-(4-methylpentanoyl)chroman (C-37): 2-(4-Methylpentanoyl)-4-(3-methylbuten-2-yl)phloroglucinol (C-1, 2 g) was suspended in benzene (25 ml). Trifluoroacetic acid (1.5 ml) was added, and the mixture was stirred for 6 h at room temperature. Chromatography of the resulting product yielded C-36 and C-37 in a ratio of about 2:3. These chromans both recrystallized from petroleum ether to give light-straw-colored crystals. C-36: mp 111 to 115°C, ^{13}C NMR (δ, $CDCl_3$ + CD_3OD), 205.9(s), 163.4(s), 160.1(s), 159.6(s), 103.9(s), 99.7(s), 94.9(d), 75.4(s), 41.2(t), 33.7(t), 31.6(t), 27.4(d), 26.4(q, 2C), 22.3(q, 2C), and 15.8(t); C-37: mp 128 to 131°C, ^{13}C NMR (δ, $CDCl_3$ + CD_3OD), 205.2(s), 164.1(s), 162.3(s), 156.5(s), 104.6(s), 99.8(s), 94.7(d), 75.6(s), 41.8(t), 33.5(t), 31.0(t), 27.6(d), 26.4(q, 2C), 22.3(q, 2C), and 16.2(t).

To distinguish between C-36 and C-37, both chromans were methylated with dimethyl sulfate to yield the two dimethoxy products 5,7-dimethoxy-2,2-dimethyl-6-isocaproyl chroman (36dc) and 5,7-dimethoxy-2,2-dimethyl-8-isocaproyl chroman (37dc), of which the ^{1}H NMR spectra were consecutively recorded in $CDCl_3$ and in C_6D_6 to observe the solvent-induced shifts of the methoxy singlets (4). In Fig. 2, both methoxy signals in the spectrum of 37dc exhibit large upfield shifts in the C_6D_6 spectrum compared with the $CDCl_3$ spectrum. Recording of the spectrum of 36dc in C_6D_6 reveals a very small upfield shift of one methoxy signal, indicating steric inhibition of solvation due to *ortho-ortho* adjacency of large substituents (thus assigned to the methoxy protons on C-5). The other signal shifts to δ3.32, a drastic upfield shift from the position of the signal in the $CDCl_3$ spectrum, representing the methoxy group in the 7-position which is flanked on one side by the aromatic proton.

5,7-Dihydroxy-2,2-dimethyl-8-butyryl chroman (C-35): Following the procedure outlined above, the reaction of C-19 with trifluoroacetic acid gave rise to two chromans, of which the chroman with the longest column retention time (silica; benzene-ethyl acetate, 9:1), 5,7-dihydroxy-2,2-dimethyl-8-butyryl chroman (C-35), was isolated in 70% yield; mp 102 to 104°C, ^{13}C NMR (δ, DMSO-d_6), 205.0(s), 164.2(s), 162.4(s), 156.6(s), 104.5(s), 99.8(s), 94.6(d), 75.7(s), 45.7(t), 31.0(t), 26.3(q, 2C), 18.3(t), 16.2(t), and 13.8(q).

5,7-Dihydroxy-2,2-dimethyl-6-nonanoyl chroman (C-38) and 5,7-dihydroxy-2,2-dimethyl-8-nonanoyl chroman (C-39): Using the procedure for the preparation of C-35, C-36, and C-37 described above, the reaction of 2-nonanoyl-4-(3-

TABLE 1. Antibacterial activity of some selected caespitin derivatives[a]

Compound	*S. aureus*		*S. pyogenes*	
	MIC	MBC	MIC	MBC
C-1	16	16	8	8
C-5	125	125	32	32
C-8	125	125	32	32
C-9	64	250	4	8
C-10	64	64	16	32
C-12	16	125	4	8
C-13	16	32	8	8
C-14	32	32	8	8
C-15	32	32	8	8
C-17	125	125	32	32
C-18	125	125	8	8
C-19	125	500	8	8
C-20	64	125	16	16
C-21	64	125	4	4
C-22	32	32	8	8
C-23	8	8	4	8
C-24	32	32	8	8
C-25	16	16	4	4
C-26	16	16	8	64
C-27	250	250	8	32
C-28	64	125	2	8
C-30	16	32	8	8
C-31	16	16	8	8
C-32	2	32	1	1
C-33	2	2	1	1
C-34	125	125	32	64
C-35	32	32	32	32
C-37	16	32	8	8
C-38	500	1,000	4	125
C-39	64	64	<1	8

[a] MIC and MBC are in micrograms per milliliter. Compounds not included are those with MIC or MBC values ≧64 μg/ml for the listed organisms. None of the compounds exhibited MIC or MBC values less than 64 μg/ml for *E. coli*, *P. mirabilis*, or *Pseudomonas aeruginosa* and can thus be regarded as inactive against these organisms.

TABLE 2. Antifungal activity of some selected caespitin derivatives[a]

Compound	*C. albicans*		*C. tropicalis*		*A. corymbifera*		*A. fumigatus*		*T. mentagrophytes*		*T. rubrum*		*S. schenkii*		*M. canis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
C-1	25	100	50	100	100	>100	100	100	6	13	6	6	25	50	6	13
C-7	100	>100	100	100	100	100	100	100	25	100	50	50	50	100	50	50
C-8	50	>100	50	>100	100	>100	100	100	50	>100	50	25	50	100	25	50
C-9	50	100	50	100	50	100	50	50	50	50	12.5	12.5	50	50	25	25
C-10	25	50	50	>100	50	50	50	50	13	13	13	13	25	25	6	6
C-12	25	25	25	25	50	50	50	>100	13	13	6	6	13	13	6	6
C-13	13	25	13	13	50	50	25	50	6	6	13	13	13	13	6	6
C-14	25	25	25	25	100	>100	100	100	13	13	25	25	25	25	13	13
C-15	13	25	13	25	25	50	50	50	13	25	13	13	25	50	6	13
C-17	50	100	50	50	100	100	100	>100	25	50	13	13	25	25	25	50
C-18	50	50	50	100	50	50	50	50	25	25	13	25	25	25	13	13
C-19	13	25	25	25	100	>100	100	>100	13	13	3	3	13	25	6	6
C-20	25	100	50	50	100	100	50	50	13	13	13	13	25	25	13	25
C-21	12.5	50	50	50	12.5	12.5	50	50	6.2	6.2	6.2	6.2	12.5	25	6.2	6.2
C-22	6.2	6.2	25	25	12.5	12.5	25	50	6.2	12.5	3.1	6.2	6.2	6.2	1.6	3.1
C-23	13	25	50	100	25	25	50	50	6	6	3	13	13	13	1.6	3
C-24	13	13	25	100	25	50	100	50	6	13	6	6	13	13	6	6
C-27	>100	>100	100	100	100	>100	100	>100	100	100	25	25	50	50	50	50
C-30	25	25	50	50	100	100	100	100	13	13	6	6	25	25	13	6
C-31	13	100	25	100	>100	>100	100	100	3	3	6	6	25	50	6	6
C-33	13	100	13	>100	6	25	100	100	6	25	6	13	6	13	3	3
C-34	25	100	50	100	50	50	25	>100	25	50	25	25	50	50	12.5	25
C-35	25	50	25	25	25	25	50	50	13	25	13	13	25	50	6	6
C-36	100	100	100	100	100	>100	100	>100	>100	100	25	25	>100	>100	100	100
C-37	100	100	100	100	100	50	100	>100	6	6	25	25	6	6	13	13
C-42	50	>100	>100	>100	>100	>100	>100	>100	>100	>100	25	25	>100	>100	>100	>100
Nystatin[b]	2	2.5	3	6	5.5	>100	9	>56	8	8	4	9	17	21	>4	>7

[a] MIC and MBC are in micrograms per milliliter. Compounds not included are those with MIC or MBC values ≧50 μg/ml for all listed organisms.
[b] Averages over four series.

methylbuten-2-yl)phloroglucinol (C-29) with trifluoroacetic acid gave rise to C-38 and C-39 after column chromatography. C-38: mp 108 to 110°C, ^{13}C NMR (δ, $CDCl_3$ + CD_3OD), 206.9(s), 163.9(s), 160.6(s), 159.0(s), 104.5(s), 101.2(s), 95.5(d), 75.9(s), 44.0(t), 32.3(t), 32.0(t), 29.7(t), 29.6(t), 29.3(t), 26.7(q, 2C), 25.3(t), 22.7(t), 16.2(t), and 14.1(q); C-39: mp 100 to 101°C, ^{13}C NMR (δ, $CDCl_3$ + CD_3OD), 206.8(s), 164.9(s), 160.9(s), 157.4(s), 106.0(s), 100.0(s), 95.5(d), 76.1(s), 44.6(t), 31.9(t), 31.6(t), 29.7(t), 29.6(t), 29.3(t), 26.8(q, 2C), 25.4(t), 22.7(t), 16.4(t), and 14.1(q). The configurations of C-38 and C-39 were determined by ^1H NMR solvent shifts of their dimethoxy derivatives as described above.

2,4-Dibutyryl-6-(propen-2-yl)phloroglucinol (C-42): 2-Butyryl-4-(propen-2-yl)phloroglucinol (C-8) was treated by the procedure described for the preparation of C-33 to yield C-42 after chromatography: mp 86 to 90°C, ^{13}C NMR (δ, DMSO-d_6), 205.8(s, 2C), 170.9(s), 168.2(s, 2C), 136.8(d), 113.8(t), 104.1(s), 103.6(s, 2C), 45.8(t, 2C), 26.1(t), 17.5(t, 2C), and 13.7(q, 2C).

RESULTS AND DISCUSSION

The synthesis of a series of caespitin (C-1)-related compounds was identified as the principal objective. Initial modification of the mother compound included both lengthening and shortening of the phenone side chain and expansion of the 3-position to include propen-2-yl, 3-methylbuten-2-yl, and benzyl moieties. To determine the effects of branching, alicyclic, and aromatic substitution and chlorination and etherification of the side chain, representatives of each of these classes of compounds were synthesized (C-9, C-11, C-14, C-15, and C-27). Compounds lacking either the phenone moiety (C-6 and C-16) or the alk-2-enyl substituents (C-3, C-4, and C-5) or both (C-2) were included in the series. Diketones (C-33, C-42, and C-43), bichromans (C-40 and C-41), chromans (C-35 through C-39), and furans (C-34) were considered structural analogs and also included.

MIC and MBC determinations. As can be seen from Tables 1 and 2, the action of these compounds is probably microbicidal, since the MICs and MBCs of most of the compounds did not differ significantly.

Based on the observation of structural features of the active and the less active compounds, several general tendencies may be observed. (i) Lengthening or branching of the phenone side chain generally seemed to enhance activity within a structural class (C-8 versus C-12 and C-13 for allyl phlorophenones; C-17 versus C-23 and C-1 for prenyl phlorophenones). Lengthening beyond a critical carbon chain length, however, seemed to lead to diminished activity (C-29). (ii) Introduction of a benzyl moiety in the place of allyl or prenyl likewise tended to enhance activity (C-30,

TABLE 3. Activity of test compounds C-13, C-24, and C-39 in two media against bacterial strains

Compound	Medium	MIC (μg/ml)		
		S. aureus NCTC 6571A	*S. aureus* ATCC 6538	*S. pyogenes* NCTC 8198
C-13	ISB	125	125	16
	BHI	32	32	4
C-24	ISB	125	125	16
	BHI	8	16	2
C-39	ISB	1	2	0.5
	BHI	1	4	0.5

TABLE 4. Initial MICs in duplicate for test and reference compounds against each of the test organisms in ISB

Compound	MIC (μg/ml)					
	S. aureus NCTC 6571A		*S. pyogenes* NCTC 8198		*C. albicans* ATCC 10231	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Nitrofurantoin	8	8	0.25	0.5		
Streptomycin	0.5	4	1	4		
Nystatin					1.6	3.2
C-1	64	64	16	16	6.4	6.4
C-31 (1st series)	125	250	8	16	12.5	6.4
C-33	4	8	1	1	12.5	6.4
C-13	64	125	16	16	12.5	6.4
C-24 (2nd series)	64	32	16	16	6.4	6.4
C-39	0.5	0.5	0.25	0.25	100	>100

C-31, and C-32). (iii) Phenone disubstitution with retention of all three phenolic hydroxyl groups seemed to drastically enhance activity (C-33). These apparent structure-activity relationships are currently being investigated further.

Antimicrobial resistance. In the series of tests with the first group of compounds (compounds C-1, C-31, and C-33) ISB was used for the bacterial strains. During the course of the experiments it was found that *S. aureus* was giving higher MICs than expected (according to Tables 1 and 2), while *S. pyogenes* was giving less vigorous growth than usual. Accordingly, a trial was designed to compare the MICs of C-13, C-24, and C-39 against *S. aureus* ATCC 6538 (as used previously), *S. aureus* NCTC 6571A, and *S. pyogenes* NCTC 8198 in both ISB and BHI broth. The results are given in Table 3.

The differences in sensitivity were due mainly to the use of ISB rather than the use of a different strain of *S. aureus*, and growth of *S. pyogenes* was improved in BHI broth (Table 3). BHI was thus used for tests with the second group of compounds. Results for the initial MIC determinations, the passages, and the cross-resistance experiments are given in Tables 4, 5, and 6. It can be readily seen from Table 5 that the resistance of *S. aureus* to streptomycin increased dramatically, while its resistance to nitrofurantoin increased marginally in the first series and much more in the second series. *S. pyogenes* did not show the same dramatic increase with streptomycin in either series of tests, and *C. albicans* showed no increase in resistance to nystatin. The organisms thus behaved as expected against the reference antimicrobial compounds. Assessment of the results with the experimental compounds was therefore made on the basis of whether the results with *S. aureus* and *S. pyogenes* approximated more closely those obtained with streptomycin or nitrofurantoin, and whether the results obtained with *C. albicans* showed greater increases in resistance with the experimental compounds than it did with nystatin.

In the first series (Table 5, experiment 1), the MICs of none of the experimental compounds showed any significant increase against either *S. aureus* or *S. pyogenes*. The MIC of nystatin for *C. albicans* remained fixed after 10 passages, and no significant increase was seen with any of the experimental compounds. In tests with the second group of compounds (Table 5, 2), the MIC of compound C-39 for *S. aureus* rose significantly within 10 passages, whereas that of C-13 and C-24 showed a moderate rise. With *S. pyogenes* the MIC of the compounds rose eightfold. With *C. albicans* the MIC of nystatin as well as of C-13, C-24, and C-39 remained constant. The increases observed in the MIC of C-13 and C-24 for both *S. aureus* and *S. pyogenes* were deemed experimentally significant, and it was thus considered desirable to include both these compounds, together with C-39, in tests for cross-resistance with other antimicrobial compounds.

In the cross-resistance studies with the passaged strains of *S. aureus*, no evidence of increased resistance to any of the antimicrobial agents was seen except with amikacin and chloramphenicol (Table 6). The strains passaged in C-13 and C-39 showed increased resistance, but since the strains passaged in streptomycin and nitrofurantoin showed a similar increase this result is extremely difficult to interpret. The passaged strains of *S. pyogenes* showed no evidence of increased resistance to any of the tested antimicrobial agents. It is notable that the strains of *S. pyogenes* passaged in both streptomycin and nitrofurantoin gave evidence of increased resistance to amikacin. Reactions of the microorganisms to passage in these compounds correlated far more closely to passage in nitrofurantoin than to passage in streptomycin.

A certain measure of success has been achieved in elucidating broad structure-activity correlations within the range of phlorophenone antimicrobial compounds reported. Several of the compounds, particularly the diphenone C-33, show significant improvement in activity against both bacteria and fungi over caespitin. None of the test compounds

TABLE 5. Pre- and postpassage MICs for compounds C-1, C-31, and C-33 in ISB (expt 1) and for compounds C-13, C-24, and C-39 in BHI (expt 2)

Expt	Compound	MIC (μg/ml)								
		S. aureus NCTC 6571A			*S. pyogenes* NCTC 8198			*C. albicans* ATCC 10231		
		Pre	Post	No. of passages	Pre	Post	No. of passages	Pre	Post	No. of passages
1	Nitrofurantoin	8	64	10	0.5	1	5			
	Streptomycin	4	8,000	10	4	4	5			
	Nystatin							3.1	3.1	10
	C-1	64	32	10	16	8	5	6.2	12.5	9
	C-31	125	32	6	16	8	5	12.5	12.5	10
	C-33	8	8	10	1	4	6	12.5	12.5	10
2	Nitrofurantoin	8	500	10	1	4	8			
	Streptomycin	16	8,000	10	32	125	10			
	Nystatin							1.6	2.0	11
	C-13	32	64	11	4	32	10	12.5	12.5	10
	C-24	8	64	10	2	32	10	6.2	12.5	10
	C-39	1	250	10	0.5	4	7	100	200	10

TABLE 6. Cross-resistance of *S. aureus* and *S. pyogenes* to several common antimicrobial agents prior to and following passage in some selected test compounds[a]

Strain and agent	Prepassage MIC (μg/ml)	MIC (μg/ml) after passage of strain in:					
		C-13	C-24	C-33	C-39	STM	NF
S. aureus NCTC 6571A							
Penicillin G	0.06	0.06	0.06		0.06	0.06	0.06
Ampicillin	0.12	0.12	0.12		0.12	0.12	0.12
Oxacillin	0.12	0.12	0.12		0.12	0.25	0.25
Gentamicin	2	8	1		2	2	2
Amikacin	8	>32	4		>32	>32	32
Tetracycline	0.12	0.5	0.12		0.12	0.12	0.25
Chloramphenicol	8	4	>32		4	4	8
Erythromycin	0.25	0.5	0.25		0.25	0.25	0.5
Cephalothin	0.25	0.5	1		0.25	0.25	0.5
Clindamycin	0.12	0.12	0.12		0.12	0.12	0.12
Cotrimoxazole	4/76	8/152	4/76		4/76	4/76	8/152
S. pyogenes NCTC 8198							
Penicillin G	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Ampicillin	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Oxacillin	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Gentamicin	4	4	2	8	0.5	16	16
Amikacin	16	8	16	8	2	>32	>32
Tetracycline	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Chloramphenicol	1	2	2	1	1	1	2
Erythromycin	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Cephalothin	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Clindamycin	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Cotrimoxazole	0.25/4.8	0.25/4.8	0.5/9.6	0.25/4.8	0.25/4.8	0.25/4.8	0.25/4.8

[a] The MICs were obtained for *S. aureus* and *S. pyogenes* against the Sensititre AP04 antimicrobial agents prior to and following passage in compounds C-13, C-24, C-33 (only *S. pyogenes*) and C-39 and the reference compounds streptomycin (STM) and nitrofurantoin (NF).

showed any evidence that microbial resistance to their activity was likely to develop rapidly. Furthermore, no evidence was found that exposure to the activity of these compounds resulted in any increase in resistance to a very wide range of commonly used antimicrobial agents.

ACKNOWLEDGMENTS

We thank Huntingdon Research Centre, England, where most of the antimicrobial screens were carried out, and E. R. Palmer (University of Pretoria) and L. Fourie (Potchefstroom University) for recording the NMR and mass spectra, respectively.

LITERATURE CITED

1. **Dekker, T. G., T. G. Fourie, M. U. Naudé, F. O. Snyckers, and C. J. Van der Schyf.** 1984. A convenient preparation of alkenylated acylphloroglucinols. S. Afr. J. Chem. **37**:74–75.
2. **Dekker, T. G., T. G. Fourie, F. O. Snyckers, and C. J. Van der Schyf.** 1983. Studies of South African medicinal plants. II. Caespitin, a new phloroglucinol derivative with antimicrobial properties from *Helichrysum caespititium*. S. Afr. J. Chem. **36**:114–116.
3. **Dekker, T. G., T. G. Fourie, F. O. Snyckers, and C. J. Van der Schyf.** 1984. ^{13}C NMR spectra of 3-substituted phlorophenone compounds. Org. Magn. Res. **22**:607–608.
4. **Fales, H. M., and K. S. Warren.** 1967. The use of benzene in separating aromatic methoxyl bands in nuclear magnetic resonance spectroscopy. J. Org. Chem. **32**:501–503.
5. **Hoesch, K.** 1915. Eine neue Synthese aromatischer Ketone. I. Darstellung einiger Phenol-ketone. Chem. Ber. **48**:1122–1133.
6. **Meikle, T., and R. Stevens.** 1978. β-Tricarbonyl compounds. I. Synthesis of the antibiotics uliginosin A, dihydrouliginosin B, and analogues thereof. J. C. S. Perkin **I**:1303–1312.
7. **Meikle, T., and R. Stevens.** 1979. β-Tricarbonyl Compounds. II. Synthesis of 5,7-dihydroxy-8-isobutyryl-2,2-dimethyl-2H-chromen and the antibiotics uliginosin B-iBiB and uliginosin B-iBiV. J. C. S. Perkin **I**:2563–2573.
8. **Riedl, W.** 1954. VI. Mitteilung über Hopfenbitterstoffe. Synthese einiger Lupulon-Analoga mit abgewandeltem Acyl-Rest. Justus Liebigs Ann. Chem. **585**:38–42.