Cannabinoids. 2. Synthesis and Central Nervous System Activities of Some B-Ring Homocannabinoid Derivatives and Related Lactones

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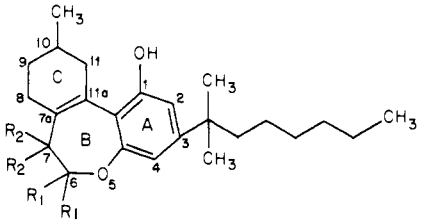
The syntheses of some novel B-ring homocannabinoid derivatives 1, 2, and 4 and related lactones 3 and 5 are described. Compounds 1–5 are 6,7,8,9,10,11-hexahydrodibenz[*b*,*d*]oxepins. CNS structure–activity correlations were determined using three rodent models. The potency and activity profile of these seven-membered ring compounds were compared with the corresponding six-membered ring homologues 6 and 7. A possible separation of CNS activities was noted with compound 1. Unexpected pharmacological activity was discovered with lactone 3, which was about equipotent to *l*-Δ^9-tetrahydrocannabinol. It was found that dimethylation at the 7 position decreased CNS activity when the 6 position was either a carbonyl or methylene group.

The previous paper in this series discussed the chemistry and CNS activities of a number of 1-amino- and 1-mercapto-7,8,9,10-tetrahydro-6*H*-dibenzo[*b*,*d*]pyrans (THDP).[1] During our studies on the synthesis of these compounds and other analogues, we envisioned the possibility of preparing some novel B-ring homocannabinoid derivatives 1, 2, and 4 and related lactones 3 and 5.[2] In



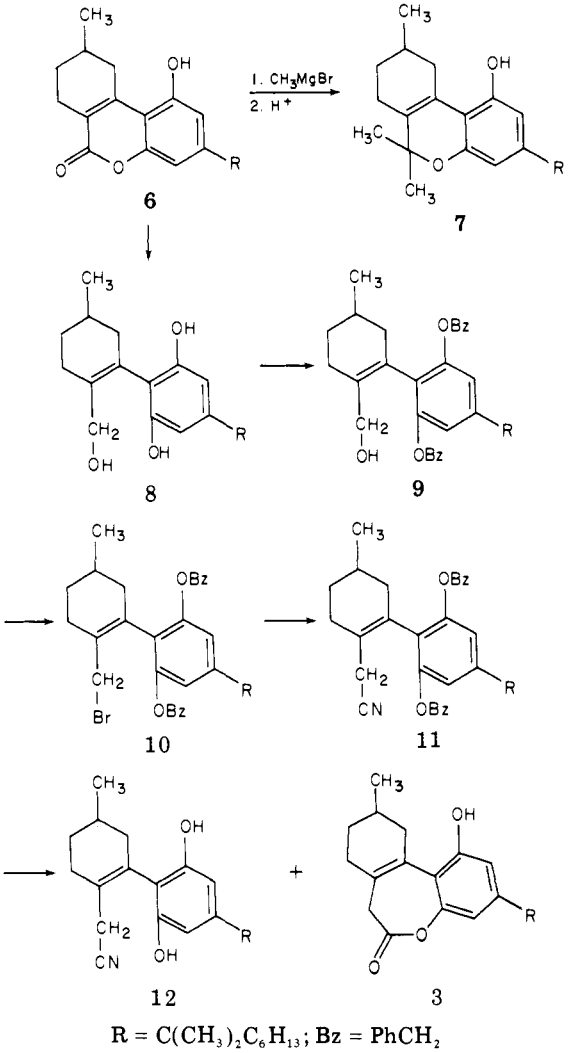
1, $R_1 = CH_3$; $R_2 = H$
2, $R_1 = R_2 = H$
3, $R_1 = =O$; $R_2 = H$
4, $R_1 = H$; $R_2 = CH_3$
5, $R_1 = =O$; $R_2 = CH_3$

this paper we wish to report the synthesis of the aforesaid compounds and their CNS activities. Some of these novel compounds were nearly as potent as the corresponding six-membered ring homologue 7 (Scheme I).

We decided to carry out our structure–activity correlations by varying the B-ring composition while keeping the 1 and 3 positions as well as the C ring constant. Studies reported by others as well as our own data have indicated the importance of a free hydroxyl group in the 1 position of THDP's.[1,3,4] Our choice of the 3-(1,1-dimethylheptyl) (1,1-DMH) side chain stems from our previous work which indicated a considerable increase in CNS potency when this group was incorporated into THDP derivatives.[1] The same conclusion regarding the relative CNS potency of 3-alkyl side chains was reported by Loev and co-workers.[3]

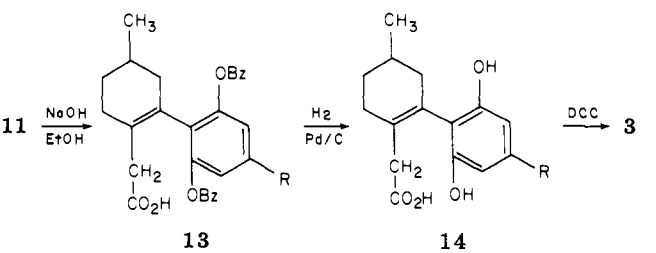
Chemistry. The approach we used toward the synthesis of B-ring homocannabinoid derivatives 1, 2, and 4 and related lactones 3 and 5 (compounds 1–5 are 6,7,8,9,10,-11-hexahydrodibenz[*b*,*d*]oxepins) hinged around the preparation of intermediate 11 as outlined in Scheme I. From our studies on analogues of 7,[1] we discovered that pyranone 6 could be reduced quantitatively with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) to triol 8.[5] Under mild alkaline conditions, 8 was readily converted to the dibenzyl-protected allylic alcohol 9 in excellent yield. Then, this was brominated, virtually quantitatively, with PBr_3 to 10, which was converted subsequently to dibenzylnitrile 11 in 89% yield using NaCN in Me_2SO.[6,7] Fearing potential reduction of the tetrasubstituted double bond by hydrogenation, our initial attempts at debenzylation of 11, using BBr_3,[8] led to low yields of desired nitrile 12 and lactone 3. Under mild hydrogenation

Scheme I



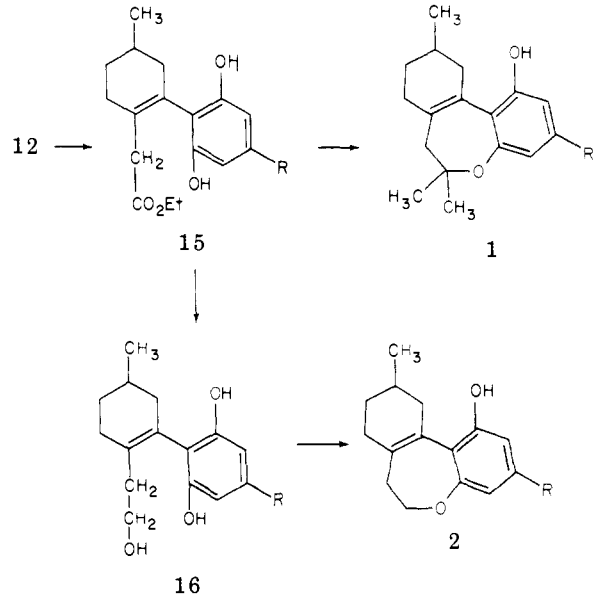
R = $C(CH_3)_2C_6H_{13}$; Bz = $PhCH_2$

Scheme II

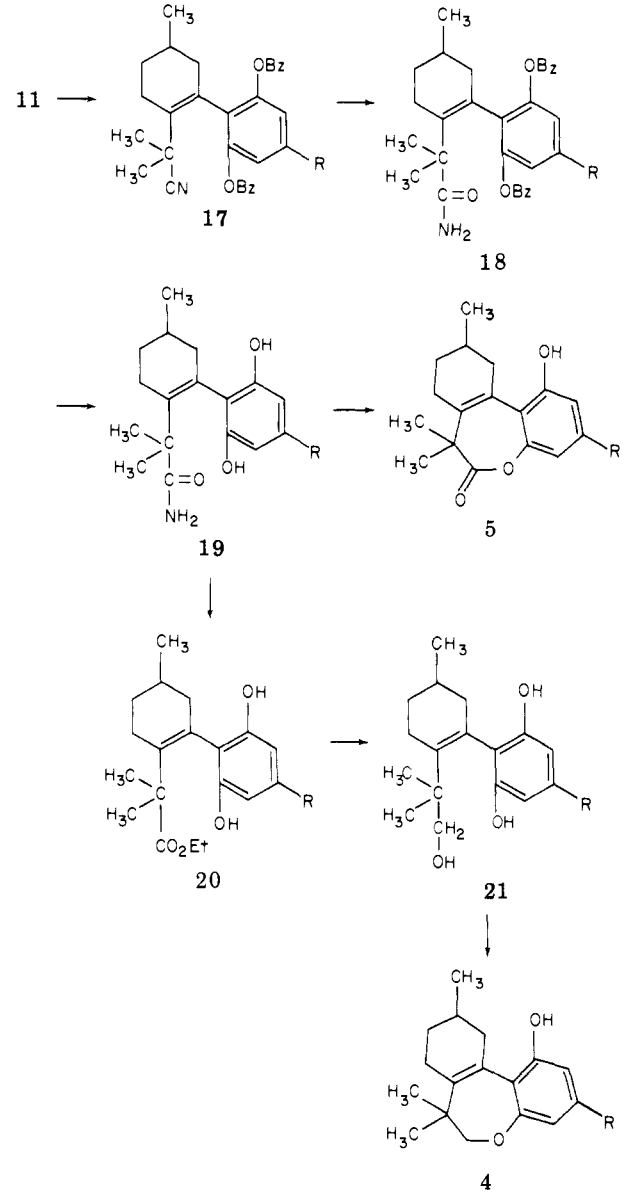


conditions, however, we found that 11 could be quantitatively converted to 12 without any evidence of double-bond reduction. The unexpected biological activity of

Scheme III



Scheme IV



3 prompted us to find a better method for its preparation (Scheme II).

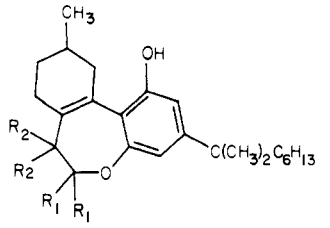
Our next objective was to prepare both analogues 1 and 2 from the common intermediate 15 according to Scheme III. Conversion of nitrile 12 to ethyl ester 15 proceeded smoothly in refluxing ethanolic HCl without any evidence of ring-closed product 3. Reaction of the ester 15 with methylmagnesium bromide afforded an intermediate triol which was treated without purification with ethanolic HCl to yield oxepin 1. Reduction of ester 15 to triol 16 was accomplished with Red-Al, in quantitative yield. Ring closure to oxepin 2 was accomplished by heating 16 neat at 110 °C with dicyclohexylcarbodiimide (DCC) according to a method developed by Vowinkel[9,10] for the preparation of ethers from simple alcohols and phenols.

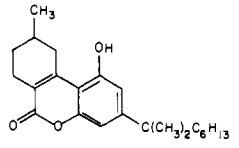
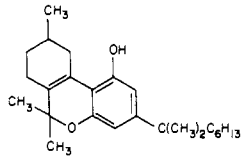
To complete our series we wished to prepare oxepins 4 and 5 according to Scheme IV. We had found that the use of KO-*t*-Bu in either HMPA or benzene, as well as NaH in THF, yielded mixtures of mono- and dimethylated products. Therefore, we decided on lithium diisopropylamide in ether and CH_3I as the methylation conditions to prepare 17. We had hoped to hydrolyze nitrile 17 under strong alkaline conditions to the corresponding acid but succeeded in preparing only the amide 18 in quantitative yield. We found, however, that after quantitative removal of the benzyl groups from 18 with 5% Pd/C, compound 19 could be cyclized thermally to the desired lactone 5. Furthermore, amide 19 was converted smoothly to ester 20 which, when reduced with Red-Al, afforded triol 21. In an analogous fashion to the preparation of 2, triol 21, when treated neat with DCC at 110 °C, afforded oxepin 4.

Structure–Activity Discussion. The three rodent test systems described in the previous publication of this series[1] were utilized in assessing the relative CNS activities of these seven-membered ring compounds. For reasons outlined previously, we initially tested these compounds at 10 mg/kg po in the septal-lesioned and muricidal rat assays and at 20 mg/kg po in the mouse activity assay. Any compound not active at these screening doses was considered inactive. An active compound was titrated down to a minimum effective dose. Hence, *l*-Δ^9-tetrahydrocannabinol (THC),[11] the major active cannabinoid in marihuana, was active in two of the three tests, whereas compound 7, which we found to be one of the most potent CNS active cannabinoids known, was active well below the screening dose in all three tests.

A summary of pharmacological testing results can be found in Table I. Since the B-ring homocannabinoid derivatives 1, 2, and 4 are homologues of 7, we felt it was appropriate to compare their biological activities with the latter reference compound. The data revealed that the most potent derivative was compound 2. It was about one-half as potent as 7 in the muricidal rat and mouse activity tests and equipotent in the septal-lesioned rat test. Both compounds appeared to possess the same profile of CNS activity. The dimethylated homologue 1 was found to be only about one-fourth as potent as reference compound 7. Of significance was the fact that, while 1 was inactive at the screening dose in the septal-lesioned rat test, it was active in the muricidal rat assay. These data would be consistent with the properties of antidepressant or appetite-suppressant drugs as opposed to an antianxiety agent. This suggestive separation of CNS activities is unique, in our experience so far, to this series of compounds. These findings may have clinical implications. Isomeric homologue 4 was inactive in both the septal-lesioned and muricidal rat assays and only minimally effective in the mouse activity test.

Table I. Pharmacological Data



Compd	R_1	R_2	Minimum effective dose: Muricidal rat[a]	Minimum effective dose: Septal-lesioned rat[a]	Minimum effective dose: Mouse act.[b]
1	CH_3	H	2.5	N[c]	2.5
2	H	H	1.25	1.25	1.25
3	=O	H	5.0	N	5.0
4	H	CH_3	N	N	20.0
5	=O	CH_3	N	N	N
6			N	N	N
7			0.62	1.25	0.62
l-Δ^9-THC[d]			5.0	N	10.0

[a] Initial testing dose, 10 mg/kg po. [b] Initial testing dose, 20 mg/kg po. [c] N = inactive at the initial testing dose. [d] Kindly supplied to us by R. Mechoulam, The Hebrew University, Jerusalem, Israel.

We were surprised to find relatively potent CNS activity for lactone **3**. Although it is only about one-eighth as potent as **7** and about equipotent to l-Δ^9-THC, it is certainly more potent than the lower homologue lactone (pyranone) **6**, which was inactive. It is interesting to note that the 7,7-dimethylated lactone **5** was inactive in all three assays. Thus, it was found that dimethylation at the 7 position decreased CNS activity when the 6 position is either a carbonyl or methylene group.

It is important to emphasize that our rodent test systems might have overlooked potentially useful CNS agents due to the low screening doses used. We selected these dose levels because many of our cannabinoids showed activity at these levels or lower.

In summary, we conclude that CNS potency nearly equal to reference compound **7** can be achieved, depending on the substitution pattern of the seven-membered B ring. Some hints of separation of CNS activities were found with compound **1**. Unexpected pharmacological activity was discovered with seven-membered B-ring lactone **3** which was about equipotent to l-Δ^9-THC.

Experimental Section

Biological Procedures. Compounds were dissolved in acetone and an equal volume of 1% Tween 80 was added. The acetone was then flash evaporated leaving a suspension of compound in 1% Tween 80 and the concentration adjusted so that the animals received the same volume per kilogram of body weight regardless of dose. Vehicle controls were run on the same "volume of injection" basis.

Detailed descriptions of the septal-lesioned, muricidal, and mouse activity tests can be found in the Experimental Section of the previous paper.[1]

Chemical Syntheses. Melting points (Thomas-Hoover capillary melting point apparatus) are uncorrected. NMR spectra were recorded on a Varian T-60 using Me_4Si as an internal standard. Mass spectra were recorded with either a Varian Mat 731 or CEC-110 instrument. Unless otherwise indicated, analytical results were within ±0.4% of theoretical values. TLC experiments were performed on 0.25-mm E. Merck precoated silica gel plates (No. 5765). Materials on plates were detected with I_2 vapor. Column chromatography procedures were performed using Woelm activity I silica gel.

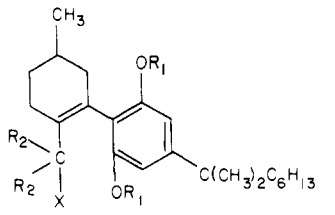
3-(1,1-Dimethylheptyl)-7,8,9,10-tetrahydro-1-hydroxy-9-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (6). This coumarin was prepared as previously described by us[1] and Adams et al.[12,13]

3-(1,1-Dimethylheptyl)-7,8,9,10-tetrahydro-1-hydroxy-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (7). This pyran was prepared as previously described by Adams et al.[12,13]

5-(1,1-Dimethylheptyl)-2-(2-hydroxymethyl-5-methyl-1-cyclohexenyl)resorcinol (8). A solution of 800 ml of benzene containing 300 ml of a 70% benzene solution of Red-Al was stirred at 0–10 °C while a suspension of 106 g (0.29 mol) of pyranone **6** and 800 ml of benzene were added portionwise over 1 h. The resultant solution was allowed to warm to room temperature and stirred an additional 2 h. Again the reaction mixture was cooled to 0–10 °C and stirred while 1400 ml of 20% HCl solution was added cautiously. The organic layer was separated and the aqueous layer extracted with ether. The combined organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford 8, 104.5 g (100%), as a white solid: NMR ($CDCl_3$–Me_2SO-d_6) δ 3.75 (s, 2 H, $-CH_2O$), 6.40 (s, 2 H, aromatic), 7.60 (broad, 2 H, phenolic OH); mass spectrum m/e 360 (M^+). (See Table II.)

5-(1,1-Dimethylheptyl)-2-(2-hydroxymethyl-5-methyl-1-cyclohexenyl)resorcinol Dibenzyl Ether (9). A solution of 61.6 g (0.17 mol) of triol **8** in 600 ml of 95% EtOH containing 27.6 g (0.20 mol) of K_2CO_3 and 46.5 g (0.369 mol) of benzyl chloride was stirred and heated at reflux for 16 h. After cooling to room temperature, 800 ml of water was added. The EtOH was removed under reduced pressure and the aqueous reaction mixture extracted several times with ether. The ethereal extracts were combined, washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford 80 g of a crude viscous oil. The

Table II. 5-(1,1-Dimethylheptyl)-2-(2-substituted 5-methyl-1-cyclohexenyl)resorcinols



Compd	R_1	R_2	X	% yield[a]	Mp, °C[b]	Formula	Analyses
8	H	H	OH	100	155-156	$C_{23}H_{36}O_3$	C, H
9	$PhCH_2$	H	OH	74	*c*	$C_{37}H_{48}O_3$	C, H
10	$PhCH_2$	H	Br	98	*c*	$C_{37}H_{47}BrO_2$	C, H, Br
11	$PhCH_2$	H	CN	89	85-86	$C_{38}H_{47}NO_2$	C, H, N
12	H	H	CN	100	116-117	$C_{24}H_{35}NO_2$	C, H, N
13	$PhCH_2$	H	CO_2H	79	69-70	$C_{38}H_{48}O_4$	C, H
14	H	H	CO_2H	100	*d*	$C_{24}H_{36}O_4$	C, H
15	H	H	CO_2Et	67	*c*	$C_{26}H_{40}O_4$	C, H
16	H	H	CH_2OH	100	*c*	$C_{24}H_{38}O_3$	C, H
17	$PhCH_2$	CH_3	CN	84	65-66	$C_{40}H_{51}NO_2$	C, H, N
18	$PhCH_2$	CH_3	$CONH_2$	99	113-114	$C_{40}H_{53}NO_3$	C, H, N
19	H	CH_3	$CONH_2$	100	*e*	$C_{26}H_{41}NO_3$	C, H, N
20	H	CH_3	CO_2Et	85	*c*	$C_{28}H_{44}O_4$	C, H
21	H	CH_3	CH_2OH	85	*c*	$C_{26}H_{42}O_3$	C, H

[a] Based on immediate precursor. [b] All solids isolated from silica gel chromatography as a single spot on TLC (10% EtOAc-PhH). [c] Isolated from chromatography as a viscous oil; single spot on TLC. [d] Isolated from chromatography as a glassy residue; single spot on TLC. [e] Isolated from chromatography as an amorphous solid; single spot on TLC.

product was purified by chromatography over 800 g of silica gel eluted with 0–4% EtOAc–PhH. The appropriate fractions were combined to afford **9**, 68.0 g (74%), as a viscous oil: NMR ($CDCl_3$) δ 3.70 (d, 2 H, 2-methylene), 5.05 (s, 4 H, benzyl methylenes), 6.60 (s, 2 H, aromatic), 7.40 (s, 10 H, benzyl aromatic); mass spectrum *m/e* 540 (M^+).

2-(2-Bromomethyl-5-methyl-1-cyclohexenyl)-5-(1,1-dimethylheptyl)resorcinol Dibenzyl Ether (10). While a solution containing 98.8 g (0.183 mol) of the dibenzyl alcohol **9** in 200 ml of anhydrous ether was being stirred at 0 °C, a solution of 59.3 g (0.22 mol) of PBr_3 in 200 ml of ether was added dropwise over a 30-min period. The reaction mixture was then heated at reflux for 1 h after which it was poured onto ice water. The resulting aqueous mixture was extracted with ether; the ether layer washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to yield **10**, 108.8 g (98%), as a viscous colorless oil. This material was used without further purification: NMR ($CDCl_3$) δ 3.85 (s, 2 H, $-CH_2Br$), 5.05 (s, 4 H, benzyl methylenes), 6.55 (s, 2 H, aromatic), 7.40 (s, 10 H, benzyl aromatic); mass spectrum *m/e* 602, 604 (M^+).

2-(2-Cyanomethyl-5-methyl-1-cyclohexenyl)-5-(1,1-dimethylheptyl)resorcinol Dibenzyl Ether (11). To a solution of 28.0 g (46.5 mmol) of resorcinol **10** in 125 ml of Me_2SO was added 4.55 g (93 mmol) of NaCN. The reaction mixture was stirred at 25 °C for 16 h after which it was poured onto 1 l. of water. The aqueous solution was extracted several times with ether; the combined ether extracts were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford a crude viscous oil. This oil was chromatographed over 500 g of silica gel eluted with benzene. Appropriate fractions were combined to yield **11**, 22.7 g (89%), as a colorless viscous oil which later solidified: NMR ($CDCl_3$) δ 2.90 (s, 2 H, $-CH_2CN$), 5.10 (s, 4 H, benzyl methylene), 6.65 (s, 2 H, aromatic), 7.45 (s, 10 H, benzyl aromatic); mass spectrum *m/e* 549 (M^+).

2-(2-Cyanomethyl-5-methyl-1-cyclohexenyl)-5-(1,1-dimethylheptyl)resorcinol (12). A solution of 8.80 g (16.0 mmol) of dibenzylnitrile **11** in 200 ml of 95% EtOH containing 440 mg of 5% Pd/C was shaken at 25 °C for 17 h at an initial hydrogen gas atmosphere of 30 psi. The reaction mixture was then filtered and the filtrate concentrated under reduced pressure affording **12**, 5.90 g (100%), as a solid: NMR ($CDCl_3$) δ 2.95 (s, 2 H, $-CH_2CN$), 4.90 (broad, s, 2 H, –OH), 6.45 (s, 2 H, aromatic); mass spectrum *m/e* 369 (M^+).

2-(2-Carboxymethyl-5-methyl-1-cyclohexenyl)-5-(1,1-dimethylheptyl)resorcinol Dibenzyl Ether (13). A reaction mixture consisting of 10.0 g (18.19 mmol) of nitrile **11**, 30 ml of 95% EtOH, and 50 ml of 50% (w/w) NaOH was stirred and heated to reflux for 16 h. Most of the EtOH was removed under reduced pressure and then 250 ml of water was added. The aqueous layer was acidified to pH 1 and the organic material extracted with ether. The ether layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford a crude solid. Purification was accomplished by chromatography over 200 g of silica gel eluted with 1–3% EtOAc–PhH to afford **13**, 8.2 g (79%), as a white solid: NMR ($CDCl_3$) δ 2.80 [s, 2 H, $-CH_2C(=O)-$], 4.95 (s, 4 H, benzyl methylene), 6.50 (s, 2 H, aromatic), 7.75 (s, 10 H, benzyl aromatic); mass spectrum *m/e* 568 (M^+).

2-(2-Carboxymethyl-5-methyl-1-cyclohexenyl)-5-(1,1-dimethylheptyl)resorcinol (14). A mixture of 7.0 g (12.3 mmol) of dibenzyl acid **13**, 150 ml of absolute EtOH, and 800 mg of 5% Pd/C was hydrogenated for 16 h at an initial pressure of 40 psi. Filtration and evaporation of the filtrate under reduced pressure yielded **14**, 4.80 g (100%), as a glassy residue: NMR ($CDCl_3$) δ 3.00 [s, 2 H, $-CH_2C(=O)-$], 6.40 (s, 2 H, aromatic), 7.05 (broad, 3 H, OH); mass spectrum *m/e* 388 (M^+), 370.

5-(1,1-Dimethylheptyl)-2-(2-ethoxycarbonylmethyl-5-methyl-1-cyclohexenyl)resorcinol (15). A solution of 15.0 g (40.59 mmol) of nitrile **12** in 400 ml of 95% EtOH was saturated with anhydrous HCl and refluxed for 16 h. TLC (10% EtOAc–PhH) revealed starting material still present; therefore, the solution was again saturated with HCl and refluxed an additional 24 h. Most of the EtOH was removed under reduced pressure and 500 ml of water was added to the residue. The aqueous mixture was extracted with ether; the ether layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford a crude gum. The desired product was purified by chromatography over 300 g of silica gel eluted with 1–2% EtOAc–PhH to yield **15**, 11.36 g (67%), as a viscous oil: NMR ($CDCl_3$) δ 2.95 [s, 2 H, $-CH_2C(=O)-$], 4.15 (q, 2 H, $-OCH_2-$), 5.50, 5.70 (s, s, 1 H, 1 H, OH), 6.45 (s, 2 H, aromatic); mass spectrum *m/e* 416 (M^+).

5-(1,1-Dimethylheptyl)-2-[2-(2-hydroxyethyl)-5-methyl-1-cyclohexenyl]resorcinol (16). A solution of 240 ml of benzene containing 120 ml of a 70% benzene solution of Red-Al was stirred at 0–10 °C while a solution of 11.30 g (27.1 mmol) of the ester **15** in 100 ml of benzene was added over a 15-min period. The resultant solution was stirred at room temperature for 1 h after which it was again cooled to 0–10 °C and carefully decomposed with 1 l. of 10% aqueous HCl. The organic layer was separated and the aqueous layer extracted with ether. The combined organic layer was washed with water, dried over Na_2SO_4, and evaporated

under reduced pressure to afford **16**, 10.15 g (100%), as a viscous oil which solidified: NMR ($CDCl_3$) δ 3.70 (t, 2 H, $-CH_2O$), 5.90 (broad, 2 H, phenolic OH), 6.45 (s, 2 H, aromatic); mass spectrum m/e 374 (M^+).

2-[2-(2-Cyano-1-methylethyl)-5-methyl-1-cyclohexenyl]-5-(1,1-dimethylheptyl)resorcinol Dibenzyl Ether (17). Lithium diisopropylamide was prepared by slowly adding 29 ml (50 mmol) of 1.72 M CH_3Li in ether to a solution of 7.0 ml (50 mmol) of diisopropylamine in 150 ml of anhydrous ether that was cooled to 0 °C. Thirty minutes after addition, a solution of 5.5 g (10 mmol) of nitrile **11** dissolved in 50 ml of ether was slowly added to the 0 °C solution. After stirring at 0 °C for 15 min, 15.5 ml (250 mmol) of CH_3I was added slowly and the temperature allowed to rise to 25 °C for 16 h. The precipitate formed dissolved upon addition of 200 ml of water. The layers were separated and the aqueous layer was extracted with ether. The combined ether layer was washed with 1 N HCl and water, dried over Na_2SO_4, and evaporated under reduced pressure to afford a gummy residue. Purification was achieved by chromatography on 200 g of silica gel eluted with benzene to yield **17**, 4.84 g (84%), as a viscous oil which later solidified: NMR ($CDCl_3$) δ 5.02, 5.05 (s, s, 4 H, benzyl methylene), 6.55 (s, 2 H, aromatic), 7.35 (m, 10 H, benzyl aromatic); mass spectrum m/e 577 (M^+).

2-[2-(1-Carboxamido-1-methylethyl)-5-methyl-1-cyclohexenyl]-5-(1,1-dimethylheptyl)resorcinol Dibenzyl Ether (18). A mixture consisting of 19.5 g (33.75 mmol) of nitrile **17**, 60 ml of 95% EtOH, and 100 ml of 50% (w/w) NaOH was stirred and refluxed for 72 h. Most of the EtOH was removed under reduced pressure and 500 ml of water added. The aqueous layer was acidified to pH 1 with 6 N HCl and extracted several times with ether. The ether layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford **18**, 19.92 g (99%), as a viscous oil which slowly solidified to a white solid: NMR ($CDCl_3$) δ 5.05, 5.10 (s, s, 2 H, 2 H, benzyl methylene), 6.60 (s, 2 H, aromatic), 7.35 (s, 10 H, benzyl aromatic); mass spectrum m/e 595 (M^+).

2-[2-(1-Carboxamido-1-methylethyl)-5-methyl-1-cyclohexenyl]-5-(1,1-dimethylheptyl)resorcinol (19). A mixture of 11.8 g (19.8 mmol) of dibenzylamide **18**, 200 ml of 95% EtOH, and 500 mg of 5% Pd/C was shaken at room temperature at an initial hydrogen pressure of 40 psi for 16 h. TLC (10% EtOAc–PhH) indicated incomplete debenzylation; therefore, an additional 500 mg of 5% Pd/C was added and hydrogenation at an initial pressure of 40 psi was carried out for an additional 24 h. After filtration and removal of the solvent under reduced pressure, **19**, 8.24 g (100%), was isolated as an amorphous solid: NMR ($CDCl_3$) δ 6.10 (broad, 4 H, $-NH_2$, OH), 6.35 (s, 2 H, aromatic); mass spectrum m/e 398 ($M^+ - 17$), which indicated loss of NH_3 upon ring closure.

5-(1,1-Dimethylheptyl)-2-[2-(1-ethoxycarbonyl-1-methylethyl)-5-methyl-1-cyclohexenyl]resorcinol (20). A solution containing 8.24 g (19.8 mmol) of amide **19** dissolved in 500 ml of 95% EtOH was saturated with gaseous HCl and the solution refluxed for 16 h. The solution was again saturated with HCl and refluxed an additional 24 h. Most of the EtOH was removed under reduced pressure and 500 ml of water added to the residue. The organic material was extracted with ether; the ether layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to afford a crude oil. Purification was accomplished by chromatography over 250 g of silica gel eluted with 1% EtOAc–PhH to yield **20**, 7.50 g (85%), as a viscous oil: NMR ($CDCl_3$) δ 3.85 (q, 2 H, $-OCH_2-$), 5.00, 5.45 (s, s, 1 H, 1 H, OH), 6.45 (s, 2 H, aromatic); mass spectrum m/e 444 (M^+).

5-(1,1-Dimethylheptyl)-2-[2-(2-hydroxy-1,1-dimethylethyl)-5-methyl-1-cyclohexenyl]resorcinol (21). A solution of 120 ml of benzene containing 60 ml of a 70% benzene solution of Red-Al was stirred at 0–10 °C while a solution of 7.0 g (15.7 mmol) of ester **20** in 50 ml of benzene was added over a 10-min period. The resultant solution was stirred at room temperature for 3 h after which it was again cooled to 0–10 °C and carefully decomposed with 500 ml of 10% HCl. The layers were separated and the aqueous layer was extracted with ether. The combined organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to yield a crude glassy residue. Purification was achieved by chromatography on 160 g of silica gel eluted with 1–2% EtOAc–PhH to afford **21**, 5.41 g (85%), as a viscous oil: NMR ($CDCl_3$) δ 3.40 (**d**, 2 H, $-CH_2O$), 6.40 (s, 2 H, aromatic); mass spectrum m/e 402 (M^+).

3-(1,1-Dimethylheptyl)-6,7,8,10,11-hexahydro-1-hydroxy-6,6,10-trimethyldibenz[*b*,*d*]oxepin (1). To a solution of 40 ml of 3 M CH_3MgBr in ether was slowly added 3.3 g (7.9 mmol) of ester **15** dissolved in 100 ml of benzene. The mixture was refluxed 16 h after which it was poured onto ice containing 15 ml of concentrated H_2SO_4. The organic material was extracted with ether; the ether was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure to yield 3.16 g of a viscous oil. According to NMR ($CDCl_3$) this was the corresponding triol: δ 4.75 (broad, s, 3 H, OH), 6.50 (s, 2 H, aromatic).

The crude triol was dissolved in 100 ml of 95% EtOH and the solution saturated with gaseous HCl. After 30 min the EtOH was removed under reduced pressure and the residue purified by chromatography on 100 g of silica gel eluted with benzene to afford **1**, 2.30 g (75%), as an amorphous solid: NMR ($CDCl_3$) δ 4.90 (s, 1 H, OH), 6.55 (s, 2 H, aromatic); mass spectrum m/e 384 (M^+). Anal. ($C_{26}H_{40}O_2$) C, H.

3-(1,1-Dimethylheptyl)-6,7,8,9,10,11-hexahydro-1-hydroxy-10-methyldibenz[*b*,*d*]oxepin (2). A mixture of 2.65 g (7.07 mmol) of alcohol **16** and 1.65 g (8.0 mmol) of DCC was melted at 110 °C for 2 h. During this time, a solution formed and after ~30 min dicyclohexylurea began to precipitate out. Most of the residue was dissolved in benzene (the urea did not dissolve) and applied to 130 g of silica gel and then eluted with benzene to yield **2**, 1.29 g (51%), as a white solid: NMR ($CDCl_3$) δ 4.35 (t, 2 H, $-CH_2O-$), 4.90 (s, 1 H, OH), 6.55 (m, 2 H, aromatic); mass spectrum m/e 356 (M^+). Anal. ($C_{24}H_{36}O_2$) C, H.

3-(1,1-Dimethylheptyl)-6,7,8,9,10,11-hexahydro-1-hydroxy-10-methyldibenz[*b*,*d*]oxepin-6-one (3). Over a 15-min period, a solution of 2.19 g (10.6 mmol) of DCC in 50 ml of CH_2Cl_2 was added to a 25 °C solution of 3.5 g (9.0 mmol) of acid **14** in 35 ml of CH_2Cl_2. After stirring an additional 1 h, the precipitate was filtered and the filtrate concentrated under reduced pressure to afford a crude glassy residue. Purification was accomplished by chromatography over 100 g of silica gel eluted with benzene to yield **3**, 2.55 g (76%), as a glassy residue: NMR ($CDCl_3$) δ 2.90 [s, 2 H, $-CH_2C(=O)-$], 6.65 (s, 2 H, aromatic); mass spectrum m/e 370 (M^+). Anal. ($C_{24}H_{34}O_3$) C, H.

3-(1,1-Dimethylheptyl)-6,7,8,9,10,11-hexahydro-1-hydroxy-7,7,10-trimethyldibenz[*b*,*d*]oxepin (4). A mixture of 4.50 g (11.17 mmol) mmol) of alcohol **21** and 2.60 g (12.62 mmol) of DCC was stirred and heated to 110–115 °C for 3 h. The residue was stirred with benzene (most of the urea did not dissolve) and purified by chromatography on 250 g of silica gel eluted with benzene to yield **4**, 2.12 g (49%), as a viscous oil: NMR ($CDCl_3$) δ 3.25 (s, 2 H, $-CH_2O$), 6.20 (s, 2 H, aromatic); mass spectrum m/e 384 (M^+). Anal. ($C_{26}H_{40}O_2$) C, H.

3-(1,1-Dimethylheptyl)-6,7,8,9,10,11-hexahydro-1-hydroxy-7,7,10-trimethyldibenz[*b*,*d*]oxepin-6-one (5). Amide **19** (3 g, 7.2 mmol) was heated neat under N_2 at 240 °C for 10 min. The dark residue was dissolved in benzene and purified by chromatography over 100 g of silica gel eluted with benzene to yield **5**, 870 mg (30%), as a glassy residue: NMR ($CDCl_3$) δ 1.45 [s, 6 H, $-C(CH_3)_2C(=O)-$], 5.60 (s, 1 H, OH), 6.60 (m, 2 H, aromatic); mass spectrum m/e 398 (M^+). Anal. ($C_{26}H_{38}O_3$) C, H.

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Semisynthetic Cephalosporins. Synthesis and Structure–Activity Relationships of Analogues with 7-Acyl Groups Derived from 2-(Cyanomethylthio)acetic Acid or 2-[(2,2,2-Trifluoroethyl)thio]acetic Acid and Their Sulfoxides and Sulfones

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The synthesis and in vitro and in vivo activities of a series of cephalosporins having side chains derived from 2-[(2,2,2-trifluoroethyl)thio]acetic acid or 2-(cyanomethylthio)acetic acid and with acetoxymethyl or 3-heterocyclic thiomethyl substituents at the 3 position are described. In both series, increasing the oxidation state of the side-chain sulfur atom from sulfide to sulfoxide/sulfone decreased the in vitro gram-positive activity, but the effect on gram-negative activity was variable and less pronounced. The protective effectiveness in mice infected with *Escherichia coli* increased as the oxidation level of the side-chain sulfur was raised from sulfide to sulfoxide/sulfone. Replacement of the 3-acetoxymethyl by a 3-heterocyclic thiomethyl group resulted in overall improvement of activity both in vitro and in vivo for all oxidation states.

Most of the cephalosporins that possess significant antibacterial activity have on the 7 position an acetamido group to which is attached a heterocyclic or aromatic ring such as phenyl, thiophene, pyridine, or tetrazole. Cephacetrile, which has a simple cyanoacetamido group at the 7 position, is an exception to this.[1] In searching for other potentially useful cephalosporin antibiotics, we have investigated analogues with relatively simple 7-acyl side chains derived from mercaptoacetic acid. Previously, we reported the broad-spectrum activities of 7-trifluoromethylthioacetamido-3-(1-methyl-1*H*-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid (SK&F 59962, cefazaflur) and closely related analogues.[2] We have also presented the synthesis and structure–activity relationships of 7-sulfonylacetamido-3-cephem-4-carboxylic acids.[3] This article extends the work contained in these earlier papers to analogues where the 7 side chains are derived from 2-(cyanomethylthio)acetic acid and 2-[(2,2,2-trifluoroethyl)thio]acetic acid. Both of these side chains are relatively simple and contain some of the structural features present in either cefazaflur or cephacetrile. The structure–activity relationships described herein compare the effects on activity of varying the oxidation states of the 7 side chain sulfur atom (sulfide, sulfoxide, sulfone) and of varying the 3-substituent (acetoxymethyl, methylthiadiazolethiomethyl, methyltetrazolethiomethyl).

Chemistry. The sulfonylcephalosporins [(**7**, **10**, **13**, **20**, **23**, **26** (Table I)] were synthesized by direct coupling of the side-chain acid to the appropriate *tert*-butyl 7-amino-3-cephem-4-carboxylate.[4] The *tert*-butyl group was subsequently removed with trifluoroacetic acid. The rest of the cephalosporins were prepared by acylating 7-aminocephalosporanic acid (7-ACA) or a 7-amino-3-heterocyclic thiomethyl-3-cephem-4-carboxylic acid. The latter were made by displacement of acetate from 7-ACA with an appropriate thiol by the widely used general procedure[5] described in the Experimental Section.

2-(Cyanomethylthio)acetic acid (**1**) was prepared by reacting mercaptoacetic acid with chloroacetonitrile (Scheme I) and characterized as its crystalline potassium salt. The free acid was coupled to *N*-hydroxysuccinimide with DCC in THF to give the activated ester **2**. When this was treated with 1 equiv of *m*-chloroperbenzoic acid, the corresponding sulfoxide **3** was obtained. Both **2** and **3** reacted readily with the triethylamine salts of the 7-amino-3-cephem-4-carboxylic acids in DMF to give cephalosporins **5**, **6**, **8**, **9**, **11**, and **12**. Attempts to oxidize **2** to the sulfone using 2 equiv of *m*-chloroperbenzoic acid were unsuccessful. Consequently, 2-(cyanomethylsulfonyl)acetic acid (**4**) was generated directly from **1** using 2 equiv of *m*-chloroperbenzoic acid. However, attempts to acylate 7-amino-3-cephem-4-carboxylic acid or the corresponding 3-heterocyclic thiomethyl derivatives with this acid via the acid chloride, mixed anhydride, or activated ester were unsuccessful and resulted only in the recovery of starting materials. For the synthesis of **7**, **10**, and **13**, this side-chain acid was coupled to the appropriate *tert*-butyl 7-amino-3-cephem-4-carboxylate using DCC. The *tert*-butyl group was removed with TFA in the presence of a scavenger such as *m*-dimethoxybenzene or acetonitrile in order to trap the *tert*-butyl cation and minimize rearrangement to other positions on the cephalosporin.

2-[(2,2,2-Trifluoroethyl)thio]acetic acid (**14**) was readily obtained by reacting 2,2,2-trifluoroethyl iodide with mercaptoacetic acid[8] (Scheme II). Treatment with thionyl chloride gave the acid chloride. Acylation of the various 7-amino-3-cephem-4-carboxylic acids in aqueous media