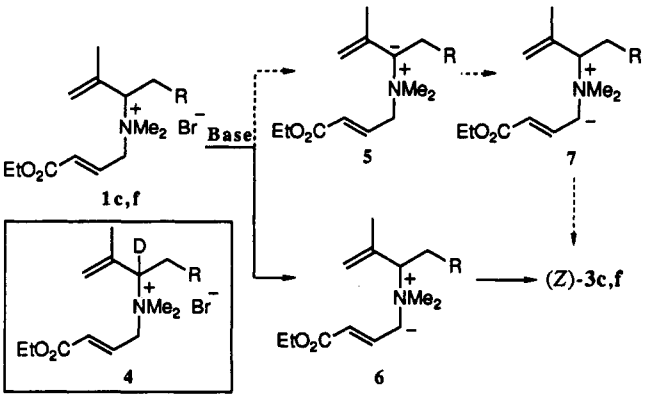
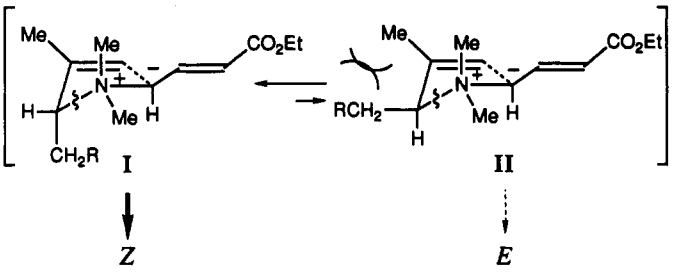
of bisallylic systems **1c** and **1f**, deuterio ammonium salt **4c** (96 ± 4% D) was synthesized and treated with potassium *tert*-butoxide in THF-HMPA at −70 °C. The deuterium content of *Z*-ester **3c** was determined to be 96 ± 4% D by ^{1}H NMR after purification by column chromatography. This result rules out allylide **5** as an intermediate and establishes the direct formation of methylide **6**. Therefore the *Z*-selective character of the present system is in marked contrast to our previous system[11] from a mechanistic standpoint.

The [2,3] sigmatropic rearrangement of **1c,f** seems to have an earlier (i.e., reactant-like) transition state than that of the stable ylides **2a,b,d,e**. Thus another envelope conformation can be postulated as a plausible transition state leading to *Z*-olefins (Scheme IV).

Scheme III



Scheme IV



The conformational preference of I over II may result from vicinal repulsion between RCH_2 and the vinyl methyl group, which Still postulated to be an important factor in the *Z*-selective Wittig rearrangement.[10,14]

(3) (a) Blackburn, G. M.; Ollis, W. D.; Plackett, J. D.; Smith, C.; Sutherland, I. O. *J. Chem. Soc., Chem. Commun.* **1968**, 186. (b) Baldwin, J. E.; Hackler, R. E.; Kelly, D. P. Ibid. **1968**, 537, 538. (c) Evans, D. A.; Andrews, G. C.; Sims, C. L. *J. Am. Chem. Soc.* **1971**, *93*, 4956. (d) Evans, D. A.; Andrews, G. C.; Fujimoto, T. T.; Wells, D. *Tetrahedron Lett.* **1973**, 1385, 1389. (e) Grieco, P. A. *J. Chem. Soc., Chem. Commun.* **1972**, 702. (f) Grieco, P. A.; Finkelforn, R. S. *J. Org. Chem.* **1973**, *38*, 2245.

(4) Cope, A. C.; Morrison, D. E.; Field, L. *J. Am. Chem. Soc.* **1950**, *72*, 59.

(5) Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. G.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4869.

(6) Snider, B. B.; Hrib, N. J.; Fuzesi, L. *J. Am. Chem. Soc.* **1976**, *98*, 7115.

(7) Grieco, P. A.; Boxler, D.; Hiroi, K. *J. Org. Chem.* **1973**, *38*, 2572.

(8) Macomber, R. S. *J. Am. Chem. Soc.* **1977**, *99*, 3072.

(9) (a) Yamamoto, Y.; Oda, J.; Inouye, Y. *J. Org. Chem.* **1976**, *41*, 303; *J. Chem. Soc., Chem. Commun.* **1973**, 848. (b) Inoue, S.; Iwase, N.; Miyamoto, O.; Sato, K. *Chem. Lett.* **1986**, 2035. Sato, K.; Miyamoto, O.; Inoue, S.; Iwase, N.; Honda, K. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1328.

(10) Still, W. C.; Mitra, A. *J. Am. Chem. Soc.* **1978**, *100*, 1927.

(11) Honda, K.; Inoue, S.; Sato, K. *J. Am. Chem. Soc.* **1990**, *112*, 1999.

(12) The stereochemistry of the rearrangement products was confirmed by ^{1}H and ^{13}C NMR ($CDCl_3$) as follows: (*E*)-**3a**, δ 1.64 (CH_3, s), 15.9; (*Z*)-**3a**, δ 1.72 (CH_3, s), 23.0; (*E*)-**3b**, δ 1.62 (CH_3, s), 16.0; (*Z*)-**3c**, δ 1.69 (CH_3, s), 23.8; (*E*)-**3c**, δ 1.59 (CH_3, s), 15.8; (*E*)-**3d**, δ 1.63 (CH_3, s), 15.9; (*E*)-**3e**, δ 1.61 (CH_3, s), 15.9; (*Z*)-**3f**, δ 1.67 (CH_3, s), 23.8; (*E*)-**3f**, δ 1.58 (CH_3, s), 16.1.

(13) (a) Baldwin, J. E.; Patrick, J. E. *J. Am. Chem. Soc.* **1971**, *93*, 3556. (b) Evans, D. A.; Andrews, G. C. *J. Am. Chem. Soc.* **1972**, *94*, 3672.

(14) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 563.

Antineoplastic Agents. 214. Isolation and Structure of Cephalostatins 7-9[1a]

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Summary: The Southern Indian Ocean marine worm *Cephalodiscus gilchristi* has been found to yield new cephalostatins 7-9.

Tube-inhabiting marine animals of the genus *Cephalodiscus* (one of two divisions in the class Pterobranchia, Hemichordata Phylum) are rarely encountered. Only some 18 species are presently known[2,3] and confined primarily to Antarctica.[4] One Southern Hemisphere temperate region species *Cephalodiscus gilchristi* was recorded[5] off the coast of South Africa in 1906 and described in more detail in 1915-17.[6] In 1988 we summarized results from the first chemical study of this genus and isolation of the powerful (P388 ED_{50} 10^{-7}-10^{-9} μg/mL) cell growth inhibitor cephalostatin 1 (**1**)[7] from *C. gilchristi*. Subsequently we described cephalostatins 2-4[8] and 5-6[9] where introduction of an aromatic C′-ring (cf. **2** corresponding to cephalostatin 6) was found to greatly reduce (P388 ED_{50} $\sim 10^{-2}$ μg/mL) the cytostatic activity. We now report that further detailed investigation of *C. gilchristi* antineoplastic

(1) (a) For series part 213 refer to: Bai, R.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1990**, 265, 17141. (b) NCI Frederick Cancer Research and Development Center, Frederick, MD 21702.

(2) Bayer, F. M. *Bull. Inst. Mar. Sci. Gulf Caribbean* **1962**, *12*(2), 306-312.

(3) Markham, J. C. *Antarctic Research Services*; Llano, G. A., Wallen, E., Eds.; University of Miami: Miami, FL, 1971; Vol. 17, p 83.

(4) The first recognized species (*C. nigrescens*) of this genus was collected during expeditions of the *Erebus* and *Terror* in 1841-2: Ridewood, W. C. *Ann. Mag. Nat. Hist.* **1912**, *10*(8) (Art. 45), 550-555.

(5) *C. gilchristi* was recognized as a new species by: Ridewood, W. C. *Mar. Invest.* **1906**, 4, 173-192.

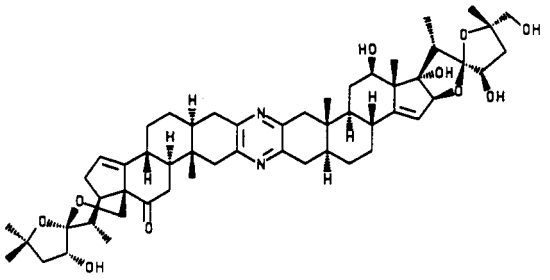
(6) (a) Gilchrist, J. D. F. *Ann. Mag. Nat. Hist.* **1915**, *16*(8) (Art. 30), 233-243. (b) Gilchrist, J. D. F. *Q. Jl. Microsc. Sci.* **1917**, *62*, 189-211.

(7) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006.

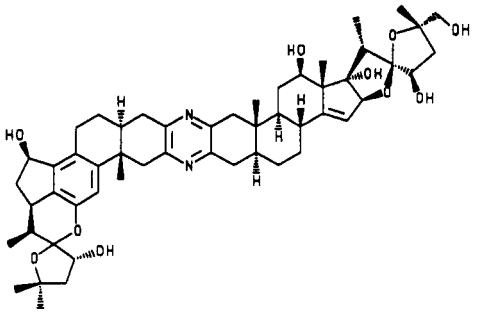
(8) Pettit, G. R.; Inoue, M.; Kamano, Y.; Dufresne, C.; Christie, N.; Niven, M. L.; Herald, D. L. *J. Chem. Soc., Chem. Commun.* **1988**, 865.

(9) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Inoue, M.; Christie, N.; Schmidt, J. M.; Doubek, D. L. *Can. J. Chem.* **1989**, 1509.

0022-3263/92/1957-0429$03.00/0



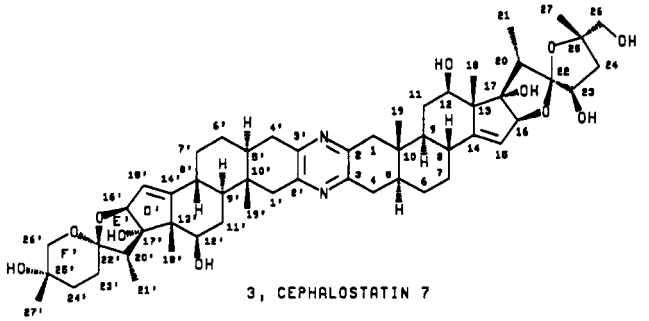
1, CEPHALOSTATIN 1



2, CEPHALOSTATIN 6

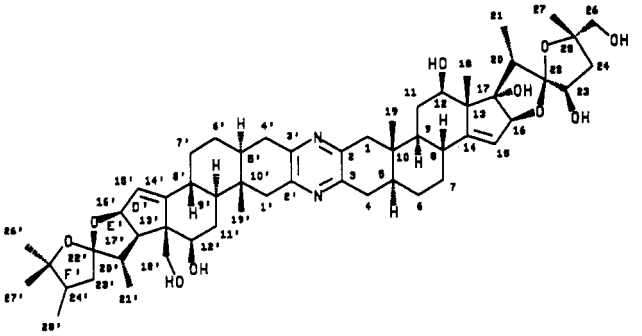
constituents has led to the discovery of three new and remarkable cephalostatins designated 7–9 that exhibit potent growth inhibitory and cytotoxic activity against diverse human solid tumor types in the U.S. National Cancer Institute's new in vitro, disease-oriented antitumor screen.[10]

A 28-g methylene chloride fraction prepared from 166 kg (wet wt) of *C. gilchristi* was separated (P388 lymphocytic leukemia cell line guided) to provide fractions h (8 mg), l (20 mg), and m (33 mg) using a series of steric exclusion and partition chromatographic procedures followed by gradient HPLC, as previously outlined.[9] Continued bioassay-directed separation of fraction m employing a gel (Sephadex LH-20) partition → HPLC → gel permeation sequence afforded (18.7 mg, 1.1×10^{-6}% yield) cephalostatin 7 (3); amorphous powder, dp 315 °C; R_f 0.39 (SiO_2) in 90:10:0.8 $CH_2Cl_2/CH_3OH/H_2O$; $[\alpha]_D$ +106° (*c* 0.244 in CH_3OH); SP-SIMS[11] found 967.5067 $[M + K]^+$ for $C_{54}H_{76}N_2O_{11}$ (calcd 967.6413 and 928.5450 for M^+); UV (CH_3OH) λ_{max} 286 (ϵ 17 400) and 310 (shoulder) nm; IR (KBr) 3430, 2960, 2920, 2880, 2860, 1715 (weak, broad), 1650–1615 (broad), 1450, 1400, 1385, 1056, 1043, and 950 cm^{-1}; for the ^1H- and ^{13}C-NMR see ref. 12.

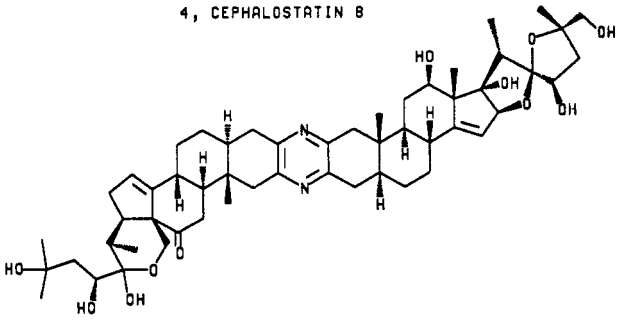


3, CEPHALOSTATIN 7

Analogous separation of fractions h and l provided (3.4 mg, 2×10^{-7}% yield and 9.2 mg, 5.5×10^{-7}% recovery, respectively) cephalostatin 8 (4) [powder, dp 313 °C; R_f 0.35 (SiO_2) in 90:10:0.8 $CH_2Cl_2/CH_3OH/H_2O$; $[\alpha]_D$ +110° (*c* 0.10 in CH_3OH); SP-SIMS[11] found 965.5261 $[M + K]^+$ for $C_{55}H_{78}N_2O_{10}$ (calcd 965.6640 and 926.5657 for M^+); UV (CH_3OH) λ_{max} 286 (ϵ 20 800), 310 (shoulder) nm; IR (KBr) 3400, 2970, 2920, 2880, 2860, 1715 (weak, broad), 1650–1600 (broad), 1445, 1400, 1385, 1037, and 965 cm^{-1}] and cephalostatin 9 (5) [powder dp 307 °C; R_f 0.38 (SiO_2) 90:10:08 $CH_2Cl_2/CH_3OH/H_2O$; $[\alpha]_D$ +105° (*c*, 0.496 in CH_3OH); SP-SIMS[12] found 929.5527 $[M + H]^+$ for $C_{54}H_{76}N_2O_{11}$ (calcd 929.5527 and 928.5450 for M^+); UV CH_3OH λ_{max} 286 (ϵ 18 600) and 310 (shoulder) nm; IR (KBr) 3400, 2990, 2930, 2880, 2860, 1710 (strong, sharp), 1645–1630 (broad), 1445, 1400, 1385, 1055, and 950 cm^{-1}].



4, CEPHALOSTATIN 8



5, CEPHALOSTATIN 9

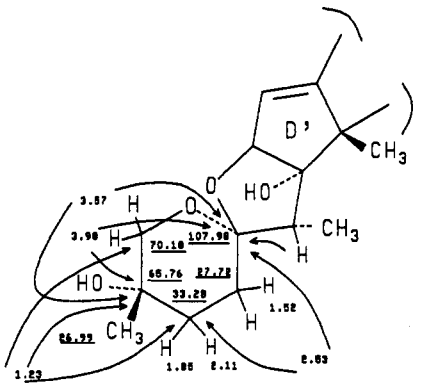
With the X-ray crystal structure[7] of cephalostatin 1 (1) and the corresponding ^1H- and ^{13}C-NMR assignments providing a solid foundation for related structural determinations we were able to assign structures 3–5 respec-

(10) Boyd, M. R.; Paull, K. D.; Rubinstein, L. R. Data display and analysis strategies from the NCI disease-oriented in vitro antitumor drug screen, in Valeriote, F. A., Corbett, T., Baker, L., Eds. *Antitumor Drug Discovery and Development*; Kluwer Academic Press: Amsterdam, 1991, in press.

(11) (a) Holzapfel, C. W.; Pettit, G. R.; Cragg, G. M. *J. Nat. Prod.* **1985**, *48*, 513–522. (b) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.* **1983**, *46*, 917–922.

(12) Bax, A.; Summers, M. A. *J. Am. Chem. Soc.* **1986**, *108*, 2094. Cephalostatin 7 (3) 400-MHz ^1H and ^{13}C NMR (pyridine-d_5, in ppm with respect to TMS; *J* values in Hz. "Right-side": ^1H 3.08 (d, 16.7, H-1a), 2.61 (H-1b), 2.89 (dd, 17.6, 4.0, H-4a), 2.66 (H-4b), 1.56 (H-5), 1.48 (H-6a), 1.20 (H-6b), 1.63 (H-7a), 1.42 (H-7b), 2.04 (H-8), 0.88 (dt, 3.5, 11.2, H-9), 2.04 (H-11a), 1.72 (q, 12.0, H-11b), 4.05 (ddd, 12.0, 5.2, 1.0, H-12), 5.64 (s, H-15), 5.24 (s, H-16), 1.31 (s, H-18), 0.75 (s, H-19), 2.83 (q, 7.0, H-20), 1.33 (d, 7.0, H-21), 4.79 (m, H-23), 2.71 (dd, 11.5, 8.0, H-24a), 2.34 (t, 11.2, H-24b), 3.79 (d, 11.2, H-26a), 3.70 (d, 11.3, H-26b), 1.61 (s, H-27); ^{13}C 46.04 (C-1), 148.86 (C-2), 148.58 (C-3), 35.78 (C-4), 41.79 (C-5), 28.27 (C-6), 28.67 (C-7), 33.79 (C-8), 53.22 (C-9), 36.32 (C-10), 28.95 (C-11), 75.59 (C-12), 55.39 (C-13), 152.73 (C-14), 122.25 (C-15), 93.16 (C-16), 91.66 (C-17), 12.56 (C-18), 11.74 (C-19), 44.50 (C-20), 9.01 (C-21), 117.17 (C-22), 71.51 (C-23), 39.53 (C-24), 82.81 (C-25), 69.28 (C-26), 26.42 (C-27). "Left-side": ^1H 3.08 (d, 18, H-1a), 2.61 (H-1b), 2.89 (dd, 17.6, 4.0, H-4a), 2.60 (H-4b), 1.56 (H-5), 1.48 (H-6a), 1.20 (H-6b), 1.55 (H-7a), 1.31 (H-7b), 2.06 (H-8), 0.98 (dt, 4.0, 11.8, H-9), 2.08 (H-11a), 1.75 (H-11b), 4.17 (ddd, 11.9, 5.0, 1.0, H-12), 5.60 (s, H-15), 5.17 (s, H-16) 1.31 (s, H-18), 0.76 (s, H-19), 2.19 (H-20), 1.26 (d, 6.8, H-21), 2.53 (dt, 4.9, 13.3, H-23a), 1.52 (H-23b), 2.11 (H-24a), 1.85 (H-24b), 3.98 (d, 11.4, H-26a), 3.57 (dd, 11.2, 1.0, H-26b), 1.23 (s, H-27); ^{13}C 46.03 (C-1), 148.86 (C-2), 148.58 (C-3), 35.78 (C-4), 41.79 (C-5), 28.27 (C-6), 28.95 (C-7), 34.02 (C-8), 52.92 (C-9), 36.32 (C-10), 29.19 (C-11), 75.66 (C-12), 56.02 (C-13), 154.85 (C-14), 119.97 (C-15), 93.74 (C-16), 93.31 (C-17), 13.00 (C-18), 11.74 (C-19), 48.45 (C-20), 8.14 (C-21), 107.90 (C-22), 27.72 (C-23), 33.28 (C-24), 65.76 (C-25), 70.18 (C-26), 26.99 (C-27). HMBC C-22′ to H-20′, H-23a′, H-26a′, H-26b′; C-24′ to H-23a′, H-27′; C-25′ to H-26a′, H-26b′, H-27′; C-26′ to H-27′.

tively to cephalostatins 7-9. While the right-side moiety of pyridizines 7-9 proved identical with that of cephalostatin 1 (1), beyond the left-side C'-ring of each were found substantial differences. Structural elucidation of cephalostatin 7 (3) primarily by high field two-dimensional ^{1}H- and ^{13}C-NMR provides an appropriate illustration. Partial structure A for rings D'-F' was derived from the



A

result of heteronuclear multiple bond correlations (HMBC).[12] Application of comparable H,H-relayed COSY,[13] NOE, and HMBC experiments to cephalostatin 8 resulted in structure 4 as most consistent with the spectral data with only the stereochemistry at C-22' remaining equivocal. The same approach was also successful with cephalostatin 9 (5) again excepting the stereochemistry at C-22'. The configuration of the C-23' hydroxyl is likely *R* as shown, and established in cephalostatin 1 (1).

Cephalostatins 7-9 (3-5) displayed remarkable potency with TI_{50} (molar) values of 10^{-9}-$<10^{-10}$ against a number of (e.g., non-small cell lung HOP 62, small cell lung DMS-273, renal RXF-393, brain U-251 and SF-295, and leukemia CCRF-CEM, HL-60, and RPM1-8226) cell lines and values of 10^{-8}-10^{-9} for the breast MCF-7 cell line; the "mean graphs" (see ref 10 for definition and interpretation) showing the patterns of relative cellular sensitivity across the panel of 60 cell lines were remarkably similar, if not essentially indistinguishable, for cephalostatins 1-4 and 7-9. By contrast, cephalostatins 5 and 6 (2) proved to be modestly cytotoxic against only two of these human cell lines (renal SN12K1 and CNS U-251) with potency reduced to GI_{50} 10^{-7}-10^{-8} molar.

Discovery of cephalostatins 1-4 and 7-9 with potent cytotoxicity against certain human cancer cell lines suggests that the pyridizine right-side unit is essential for such biological activity. Minor configuration and substitution (including an additional methyl in cephalostatin 8) alterations in the left-side E'- and F'-rings appear to have little influence on cytotoxic activity, but aromatization of the C'-ring with concommitant bonding to the side-chain system (cf. 2) markedly diminishes the potency. Perhaps cephalostatins 5 and 6 (2) represent a biosynthetic misadventure in the long evolutionary history[14] of *Cephalodiscus*.

Acknowledgment. For support of this research our appreciation is extended to the Fannie E. Rippel Foundation, the Arizona Disease Control Research Commission, Grant CA-16049-05-12 and OIG Grant CA44344-01A1 awarded by the NCI, DHHS, Virginia Piper, Eleanor W. Libby, the Donald Ware Waddell Foundation, the Robert B. Dalton Endowment, and the Fraternal Order of Eagles Art Ehrmann Cancer Fund. Also, we are pleased to thank Drs. F. M. Hogan, K. Pandel, and M. Suffness, Messrs. P. J. Daschner, and L. Williams, the National Science Foundation for equipment Grant CHE-8409644, and the NSF Regional Instrumentation Facility in Nebraska (Grant CHE-8620177).

Supplementary Material Available: 400-MHz ^{1}H-NMR spectra of Cephalostatins 7-9 (6 pages). Ordering information is given on any current masthead page.

(13) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.

(14) Eaton, T. H. *J. Paleontol.* **1970**, *44*, 969.

Dependence of Aggregation on the Basicity of Some Cesium Enolates in THF[1]

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Received September 10, 1991

Summary: Equilibrium constants in THF for dimerization of several cesium enolates of benzylic ketones show a decrease as the ion-pair acidities of the parent ketones increase when steric factors are comparable.

Many enolate salts are known to be aggregated in synthetically important ethereal solvents such as THF.[2-4] With increasing delocalization of the carbanionic charge the enolate ion becomes less effective in solvating the cation compared to solvent dipoles and aggregation is expected to be less important. This principle has been established qualitatively[5] but not quantitatively. Arnett and Moe[4] have recently shown that aggregation numbers of some lithium enolates and related phenolates depend also on steric bulk and the type of acidic group. In the present work we present the first determinations of equilibrium constants for dimerization of some cesium enolates and show that the principle is established quantitatively for several ketones when steric effects are approximately constant.

The ketones selected for study are the aryl-substituted acetones, 1,3-diphenyl- (1), 1,3-di(1-naphthyl)- (2),[6] 1,3-di(4-biphenylyl)- (3),[7] and 1,1,3,3-tetraphenylacetone (4),[8]

(1) Carbon Acidity. 81. For part 80 see: Streitwieser, A.; Dixon, R. E.; Williams, P. G.; Eaton, P. E. *J. Am. Chem. Soc.* **1991**, *113*, 357-8.

(2) Jedlinski, Z.; Misiolek, W.; Glowkowski, W. *Synlett* **1990**, 213. Arnett, E. M.; Palmer, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 7354. Arnett, E. M.; Fisher, F. J.; Nichols, M. A.; Ribeiro, A. A. *J. Am. Chem. Soc.* **1990**, *112*, 801. For a recent review see: Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1624.

(3) Kaufmann, M. J.; Streitwieser, A. *J. Am. Chem. Soc.* **1987**, *109*, 6092.

(4) Arnett, E. M.; Moe, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 7288.

(5) Kaufman, M. J.; Gronert, S. V.; Bors, D. A.; Streitwieser, A. *J. Am. Chem. Soc.* **1987**, *109*, 602.

(6) Johnston, L. J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 6368. Coan, S. B.; Becker, E. I. In *Organic Syntheses*; Rabjohn, N., Ed.; Wiley: New York, 1963; Collect. Vol. IV, pp 174, 176.

0022-3263/92/1957-0431$03.00/0