New and Unusual Sesquiterpenes: Kelsoene, Prespatane, Epi-γ-gurjunene, and T-Cadinthiol, from the Tropical Marine Sponge *Cymbastela hooperi*

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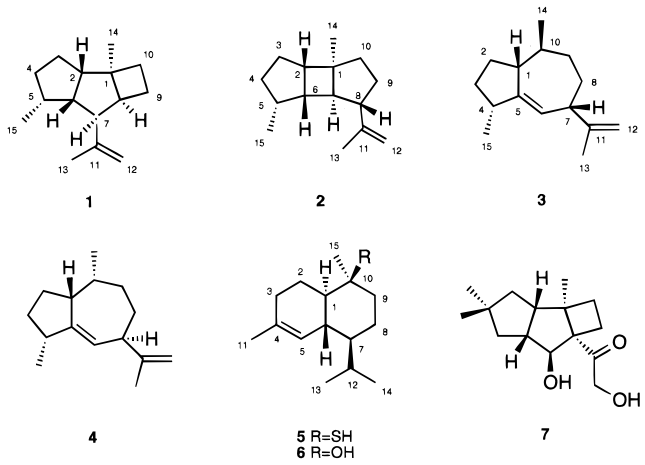
From the dichloromethane solubles of the tropical marine sponge *Cymbastela hooperi* were isolated four novel terpenoid metabolites. One of these, (1*R**,2*S**,5*R**,6*R**,7*S**,8*R**)-1,5-dimethyl-7-(1′-methylethenyl)-tricyclo[6.2.0.3^{2,6}]decane (kelsoene, **1**), possesses a most unusual carbocyclic skeleton and is the first member of a new class of sesquiterpenes, the kelsoanes. The remaining three compounds, (1*R**,2*S**,5*R**,6*R**,7*R**,8*S**)-1,5-dimethyl-8-(1′-methylethenyl)tricyclo[5.3.0.2^{2,6}]decane (prespatane, **2**), (1*R**,4*R**,7*S**,10*S**)-4,10-dimethyl-7-(1′-methylethenyl)bicyclo[5.3.0]dec-5-ene (epi-γ-gurjunene, **3**), and (1*R**,6*R**,7*S**,10*S**)-4,10-dimethyl-7-(1′-methylethyl)-10-mercaptobicyclo[4.4.0]-dec-4-ene (T-cadinthiol, **5**), are unusual natural products, particularly as sponge metabolites. All structures and their relative configurations were established by spectroscopic methods, particularly ^{1}H−^{1}H and ^{1}H−^{13}C shift correlated 2D NMR spectroscopy and accurate mass measurement (HREIMS). Compound **5** has weak *in vitro* antimalarial activity.

Introduction

Cymbastela hooperi Van Soest, Desqueyroux-Faundez, Wright, König (Axinellidae, Halichondrida), a sponge collected from Kelso reef, Great Barrier Reef, Australia, is a new sponge species belonging to a genus recently erected by Hooper and Bergquist.[1,2] With this species the number of *Cymbastela* species has increased to eight, all of which are endemic to the Australasian region. From this particular sample, compounds with potent activity toward the malaria parasite *Plasmodium falciparum* have already been isolated.[3] In order to establish a basis for chemotaxonomic comparisons between different species of *Cymbastela* and with the morphologically and chemically closely related sponge *Amphimedon terpenensis*, a detailed chemical investigation of fractions not studied during bioassay-guided isolation of antimalarial metabolites was undertaken. The results of this investigation are the basis of the current paper.

Assessment, by ^{1}H NMR and TLC, of all VLC fractions that were found to be biologically inactive in our original study[3] indicated VLC fraction 1 to be the only one that contained compounds that were not either ubiquitous lipids, sterols, or pigments. After repeated HPLC separations of this VLC fraction the four sesquiterpenes **1**−**3** and **5** were isolated.

Compound 1. The molecular formula of **1** was determined to be $C_{15}H_{24}$ by accurate mass measurement. As there were only resonances for a single carbon−carbon double bond in the ^{13}C NMR spectrum of **1** (109.8 (t), 145.6 (s) ppm), and for no other multiple bonds, it was thus evident that the molecule must be tricyclic. After association of all carbon signals with the corresponding signals for directly bonded protons, *via* an HMQC spectral measurement (see Tables 1 and 2), ^{1}H−^{1}H COSY and



HMBC spectra were recorded. The COSY spectrum allowed a continuous chain of ^{1}H−^{1}H coupling from H_2-12 and H_3-13 to H_3-14, *via* H-5 in one direction, and to H_2-10, *via* H-8 in the other to be discerned. Hence, H_2-12 and H_3-13 demonstrated allylic coupling to each other, as did H_2-12 and H-7, in turn H-7 coupled with H-6, whose resonance further correlated with resonances for H-2 and H-5. H-5 coupled with H_3-15 and H_2-4, which in turn coupled to H_2-3, whose resonances also had cross peaks with the resonance for H-2. H-2 showed a clear long-range coupling to H_3-14, delineating the first major molecular fragment of **1**. The second part of the molecule was deduced from cross peaks in the COSY spectrum between signals for H-7 and H-8 (not a completely unambiguous coupling as the resonances for these two protons are to a large extent degenerate), H-8 and H_2-9, and finally H_2-9 and H_2-10. Thus, from the ^{1}H−^{1}H coupling data the partial structure shown in Scheme 1 could be delineated and substantiated by both HMBC and INADEQUATE correlations. The remaining connectivities between C-1 and C-8, and C-1 and C-10 followed by deduction, and were confirmed by HMBC correlations observed between the resonance for H_3-14 and those for C-1, C-2, C-8, and C-10. From the NOESY spectrum of

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1997.

(1) Hooper, J. N. A.; Bergquist, P. R. *Mem. Queensl. Mus.* **1992**, *32,* 99−137.

(2) Van Soest, R. W. M.; Desqueyroux-Faundez, R.; Wright, A. D.; König, G. M. *Bull. Inst. R. Sci. Nat. Belg.*, **1996**, *66* suppl., 103−108.

(3) König, G. M.; Wright, A. D.; Angerhofer, C. K. *J. Org. Chem.* **1996**, *61,* 3259−3267.

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Scheme 1

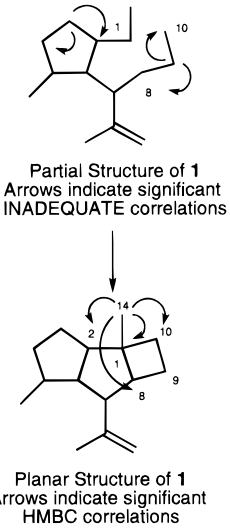


Table 1. ^{13}C NMR (75.5 MHz, $CDCl_3$) Data for Compounds 1−5

carbon	1	2	3	4	5
1	45.7 s[a]	42.1 s	44.5 d	45.8 d	49.8 d
2	57.8 d	45.3 d	28.8 t	30.0 t	24.3 t
3	26.0 t	28.1 t	31.0 t	33.8 t	30.7 t
4	33.2 t	34.7 t	30.8 d	41.0 d	134.3 s
5	36.3 d	36.8 d	140.2 s	150.2 s	122.3 d
6	49.9 d	38.5 d	125.9 d	122.4 d	38.4 d
7	48.1 d	43.8 d	51.3 d	47.5 d	46.8 d
8	47.4 d	50.3 d	32.0 t	25.6 t	20.8 t
9	14.6 t	28.2 t	35.4 t	33.2 t	43.5 t
10	33.0 t	41.7 t	40.3 d	33.8 d	49.0 s
11	145.6 s	145.3 s	147.9	145.8 s	23.8 q
12	109.8 t	109.2 t	111.5 t	110.9 t	26.2 d
13	24.1 q	23.4 q	21.9 q	21.1 q	15.1 q
14	23.5 q	20.3 q	20.1 q	15.4 q	21.4 q
15	17.7 q	14.3 q	22.1 q	19.6 q	33.0 q

[a] Multiplicity by DEPT. All assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY, NOESY).

1 cross peaks between the signals for H_3-14 and H_2-3, and H-2 and H_β-10, clearly defined the molecule as having a step/chair conformation. Further, cross peaks between the resonance for one of the protons at C-12 and the resonances for H-5, H-6, and H_3-15, and the fact that $J_{5,6}$ is approximately 7.0 Hz, positioned the isopropylene moiety (C-11 to C-13), H-5, and H-6 on the same side of the molecule. Thus, compound **1** is best described as (1*R**,2*S**,5*R**,6*R**,7*S**,8*R**)-1,5-dimethyl-7-(1′-methylethenyl)tricyclo[6.2.0.3^{2,6}]decane, for which the trivial name kelsoene is proposed.

Compound 2. By accurate mass measurement the molecular formula of **2** was determined to be $C_{15}H_{24}$. The ^{13}C NMR spectrum of **2** contained resonances for a single carbon−carbon double bond (109.2 (t), 145.3 (s) ppm), and for no other multiple bonds; **2** is thus tricyclic. A HMQC measurement allowed the association of all carbon signals with the corresponding resonances for directly bonded protons (see Tables 1 and 2). Subsequently ^{1}H−^{1}H COSY and HMBC spectra were recorded and made it possible to discern a continuous chain of ^{1}H−^{1}H coupling around the complete molecule and across the two bridges. Thus, cross peaks observed between the resonances for H_2-12 and H_3-13 indicated them to have homoallylic coupling, as do H_3-13 and H_2-12 with H-8. The resonance for H-8 has cross peaks with those for H_2-9 and H-7, with the latter proton coupling to both H_3-14 (long-range) and

Table 2. ^{1}H NMR (300 MHz, $CDCl_3$) Data for Compounds 1−5[a]

proton	1	2	3	4	5
1	–	–	1.56 (m)	2.81 (m)	1.08 (ddd, *J* = 2.4, 9.9, 11.1 Hz)
2	2.09 (ddd, *J* = 6.7, 8.9, 9.1 Hz)	2.04 (m)	1.12 (m), 2.01 (m)	1.54 (m), 1.68 (m)	1.45 (m), 1.83 (m)
3	1.35 (m), 1.47 (m)	1.41 (m), 1.68 (m)	1.72 (m), 0.98 (m)	1.23 (m), 1.68 (m)	1.98 (m)
4	1.34 (m), 1.76 (m)	1.69 (m), 1.28 (m)	2.07 (m)	2.50 (m)	–
5	2.27 (m)	1.76 (m)	–	–	5.51 (br s)
6	2.87 (ddd, *J* = 6.7, 6.8, 10.5 Hz)	1.99 (m)	5.08 (br ddd, *J* = 1.6, 1.8, 3.3 Hz)	5.39 (ddd, *J* = 2.5, 2.5, 5.8 Hz)	2.01 (m)
7	2.37 (m)	1.92 (m)	2.49 (br d, *J* = 12.8 Hz)	2.89 (m)	0.99 (m)
8	2.42 (m)	2.41 (br ddd, *J* = 5.1, 5.1, 11.1 Hz)	1.44 (m), 1.68 (m)	1.68 (m), 1.80 (m)	1.46 (m), 1.52 (m)
9	1.52 (m), 1.68 (m)	1.68 (m), 1.97 (m)	1.19 (m), 1.73 (m)	1.39 (m), 1.80 (m)	1.50 (m), 1.83 (m)
10	1.64 (m), 1.68 (m)	1.58 (m), 1.41 (m)	1.14 (m)	1.92 (m)	–
11	–	–	–	–	1.67 (br s)
12	4.80 (br s), 4.87 (br s)	4.72 (br s), 4.79 (br s)	4.73 (br ddd, *J* = 0.8, 0.9, 2.6 Hz) 4.89 (br ddd, *J* = 1.4, 1.4, 2.6 Hz)	4.74 (m), 4.76 (m)	2.17 (dqq, *J* = 3.2, 6.9, 6.9 Hz)
13	1.61 (br s)	1.67 (br s)	1.73 (br s)	1.71 (br s)	0.81 (d, *J* = 6.9 Hz)
14	1.17 (s)	0.94 (s)	0.93 (d, *J* = 6.1 Hz)	0.84 (d, *J* = 6.9 Hz)	0.91 (d, *J* = 6.9 Hz)
15	0.91 (d, *J* = 6.8 Hz)	0.87 (d, *J* = 6.7 Hz)	0.92 (d, *J* = 7.1 Hz)	1.00 (d, *J* = 7.1 Hz)	1.40 (s)

[a] All assignments are based on extensive 1D and 2D NMR experiments, including COSY90, HMQC, HMBC, HETCOR, NOE difference, and NOESY.

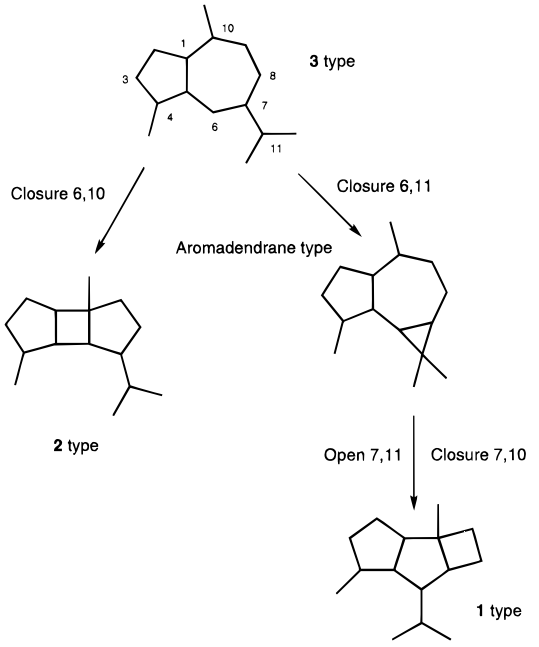
H-6. H-6 in turn couples with H-2 and H-5, the latter of which demonstrates coupling to H_3-15 and H_2-4. The protons of the methylene group further couple to H_2-3, which in turn couple to H-2, thus completing the first five-membered ring. H-2 long-range couples to CH_3-14 which is clearly bonded to C-1. From the HMBC spectrum it is also evident that C-1 and C-7 are directly bonded, completing the four-membered ring. The two protons on C-10 intercouple and further couple to H_2-9, completing the second five-membered ring and thus the planar structure of **2**. From the NOESY spectrum of **2**, cross peaks between the signals for H-8 and H-6 fixed the three rings in a step or chair conformation, while NOE interactions between CH_3-15, CH_3-14, CH_2-12 and H-7 positioned all of these atom groupings on the same side of the molecule. Compound **2** is thus (1*R**,2*S**,5*R**,6*R**,7*R**,8*S**)-1,5-dimethyl-8-(1′-methylethenyl)tricyclo[5.3.0.$2^{2,6}$]decane or prespatane.

Compound 3. Mass spectral analysis of **3** indicated it to have the molecular formula $C_{15}H_{22}$. The ^{13}C NMR spectrum of **3** is consistent with the presence of two carbon−carbon double bonds (111.5 (t), 125.9 (d), 140.2 (s), 147.9 (s) ppm), and no other multiple bonds; the molecule is thus bicyclic. In a similar fashion to that already described for compounds **1** and **2**, it was possible from the $^1H-^1H$ COSY spectrum of **3** to discern a continuous chain of $^1H-^1H$ coupling around a major part of the molecule starting from H_2-12 and H_3-13 and ending at H_3-15. Thus, cross peaks observed between the resonances for H_2-12 and H_3-13 indicated them to have homoallylic coupling, as do H_3-13 and H_2-12 with H-7. The resonance for H-7 shows cross peaks with those for H_2-8 and H-6, the latter proton also coupling to both of the protons at C-8 (long-range). H_2-8 in turn couples with H_2-9, which further couples to H-10. The methine proton H-10 further couples to H_3-14 and H-1, the latter of which couples to H_2-2, which then couples to H_2-3. The spin system is finally completed by the coupling of H_2-3 with H-4, and H-4 with H_3-15. Clearly, the neighboring carbon of C-6 must be C-5 (140.2 (s) ppm), and the two remaining carbon-carbon bonds, not as yet delineated in the $^1H-^1H$ spin network, must be between C-5 and C-1, and C-5 and C-4, giving rise to a hydroazulenoid-based molecule. Literature searches for molecules having the same basic structure as **3** led to γ-gurjunene (**4**).[4,5] Comparison of the optical rotation[4] and ^{13}C NMR data[5] for γ-gurjunene with those of **3** indicated the two molecules to be stereoisomers. As no ^1H NMR or NOE data were available for **4** these data were recorded for both **3** and **4** (see Table 2). For a commercially available sample of **4**, HMQC and HMBC spectra were also recorded to enable assignment of its ^1H NMR data (see Table 2). NOE difference measurements made with **4** showed H_3-14 to have NOE interactions with H-2α, H-8α, and H-9α, and H_2-12 to have NOEs with H-1, H-4, H-6, and H-7, clearly defining the relative configuration of this molecule to be as shown by **4**. From the NOESY spectrum of **3** a cross peak between the resonances for H-1 and H-7 indicated them to be on the same side of the molecule. Further, a cross peak between the signals for H_3-15 and H-10 revealed them both to be α oriented. Compound **3** (epi-γ-gurjunene) is thus the new natural product (1*R**,4*R**,7*S**,10*S**)-4,10-dimethyl-7-(1′-methylethenyl)-bicyclo[5.3.0]dec-5-ene.

(4) Ehret, C.; Ourisson, G. *Tetrahedron* **1969**, *25*, 1785−1799.

(5) Faure, R.; Gaydou, E. M.; Rakotonirainy, O. *J. Chem. Soc., Perkin Trans. 2* **1987**, 341−344.

Scheme 2. Proposed Biosynthetic Relationship between 3 Type and 1 and 2 Types



Compound 5. The positive EIMS of **5** contained the $[C_{15}H_{24}]^+$ fragment ion as the base peak but no other obvious ions (>1%) at higher mass. Consequently, an elemental analysis was required to establish the molecular formula as $C_{15}H_{26}S$, which was later confirmed by an accurate mass measurement of the corresponding molecular ion. Of the three elements of unsaturation implied by the molecular formula of **5**, only one was present in the form of multiple bonds, a carbon−carbon double bond (122.3 (d), 134.3 (s) ppm), indicating **5** to be bicyclic. A $^{13}C-^{13}C$ COSY measurement (INADEQUATE) made with **5** (see Supporting Information) established its basic structure unambiguously as a C-10 substituted, $\Delta^{4,5}$ cadinane derivative, e.g., **6**.[6] On the basis of the mass spectral data the functional group at C-10 was concluded to be a free thiol [S*H*, exchangeable resonance at δ 1.30 (s)], a functionality only rarely encountered in marine natural products. The two rings were deduced to be *trans*-fused on the basis of the small coupling between H-5 and H-6.[9] Further, NOE interactions between H-1, H-7, and H_{ax}-9 indicated these three protons to be on the same side of **5**, while interactions between the thiol proton and H-6 gave the thiol function the axial and β orientation. Hence, compound **5** is (1*R**,6*R**,7*S**,10*S**)-4,10-dimethyl-7-(1′-methylethyl)-10-mercaptobicyclo[4.4.0]-dec-4-ene for which the trivial name T-cadinthiol is proposed.[7]

Discussion

Kelsoene (**1**) is without precedent in the natural products literature, and its carbon skeleton 1,5-dimethyl-7-(1′-methylethenyl)tricyclo[6.2.0.$3^{2,6}$]decane (kelsoane) represents a new class of sesquiterpenes. The only other natural compound known to possess a tricyclo[6.2.0.$3^{2,6}$]-

(6) Borg-Karlson, A-K.; Norin, T.; Talvitie, A. *Tetrahedron* **1981**, *37*, 425−430.

(7) In a recent paper[2] and a review[13] we previewed the structure of **5** presented in this paper. On the basis of additional detailed NOE work, the configuration at C-10 in **5** is now revised. The configuration shown at C-5 in **1** was recently[2] incorrectly drawn and should be as shown in this work.

decane-based ring system is sulcatine G (**7**), also a sesquiterpene, reported from a laboratory-cultured slime mold,[8] but clearly elaborated through different biosynthetic cyclizations to that of **1**.

As a sesquiterpene hydrocarbon, **2** is structurally unprecedented in the marine literature, although not in the natural products literature. Two sesquiterpenes having the same skeleton are reported from the essential oil of *Geranium bourbon*.[9−11] A series of diterpenes based on this ring system have also been isolated from brown algae.[12] There are, however, no reports in the sponge related literature of any such compounds.

Biosynthetically, compounds **1** and **2** can be considered as deriving from a common precursor having the same planar structure as **3** (Scheme 2).

Compound **5** showed weak antiprotozoal activity toward cultured *Plasmodium falciparum*, clone D6 (IC_{50} = 3.6 μg/mL compared to IC_{50} = 0.4 ng/mL for artemisinin).

Experimental Section

General Experimental Procedures. For remaining details, see refs 3, 13.

Materials. See ref 3.

Extraction and Isolation. See ref 3. Repeated normal phase HPLC separations of VLC fraction 1 employing hexane as eluent afforded four sesquiterpenes, **1**−**3** and **5**.

Compound 1. Kelsoene (61.1 mg, 0.044%): a clear oil; $[\alpha]_D$ +78.1° (*c* 1.98, $CHCl_3$): EIMS *m*/*z* (%RA) 204 (M^+, 26), 189 (55), 176 (96), 161 (35), 147 (20), 135 (100), 119 (25), 105 (32); HREIMS *m*/*z* 204.1869 (M^+) ($C_{15}H_{24}$, mmu error Δ 1.0); IR (KBr) ν_{max} 3085, 2900, 1650, 1450, 1375, 885 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 1.

Compound 2. Prespartane (5.5 mg, 0.004%): a clear oil; $[\alpha]_D$ −38.0° (*c* 0.55, $CHCl_3$): EIMS *m*/*z* (%RA) 204 (M^+, 15), 189 (27), 175 (10), 163 (40), 161 (30), 147 (12), 122 (50), 107 (100), 93 (30), 81 (35); HREIMS *m*/*z* 204.1854 (M^+) ($C_{15}H_{24}$, mmu error Δ 2.5); IR (KBr) ν_{max} 3085, 2900, 1650, 1450, 1375, 885 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 1.

Compound 3. Epi-γ-gurjunene (1.1 mg, 0.0008%): a clear oil; $[\alpha]_D$ +34.6° (*c* 0.11, $CHCl_3$): EIMS *m*/*z* (%RA) 204 (M^+, >1), 189 (2), 159 (10), 145 (15), 129 (15), 119 (25), 105 (45), 91 (60); HREIMS *m*/*z* 204.1864 (M^+) ($C_{15}H_{24}$, mmu error Δ 1.5); IR (KBr) ν_{max} 3085, 2900, 1650, 1450, 1375, 885 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 1.

Compound 4. γ-Gurjunene: purchased from Fluka; ^1H NMR (300 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 1.

Compound 5. T-Cadinthiol (124.7 mg, 0.091%): a clear oil; $[\alpha]_D$ −24.3° (*c* 1.06, $CHCl_3$): EIMS *m*/*z* (%RA) 238 (M^+, >1), 204 (65), 189 (15) 161 (100), 134 (15), 119 (15), 105 (22); HREIMS *m*/*z* 238.1735 (M^+) ($C_{15}H_{26}S$, mmu error Δ 0.2); IR (KBr) ν_{max} 2900, 1450, 1375 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 1.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **1**−**5**, ^1H−^1H COSY and NOESY spectra of **1**, NOE difference spectra of **4**, and an INADEQUATE spectrum of **5**, all recorded in $CDCl_3$, using a Bruker AMX300 spectrometer (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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