Caldariellaquinone, a Unique Benzo[*b*]thiophen-4,7-quinone from *Caldariella acidophila*, an Extremely Thermophilic and Acidophilic Bacterium

By **Mario De Rosa, Salvatore De Rosa, Agata Gambacorta,** and **Luigi Minale,*** Laboratorio per la Chimica di Molecole di Interesse Biologico del C.N.R., Arco Felice, Napoli, and Istituto di Chimica Organica, Università de Catania, Viale A. Doria, Catania, Italy
Ronald H. Thomson and **Roger D. Worthington,** Department of Chemistry, University of Aberdeen, Meston Walk, Old Aberdeen AB9 2UE, Scotland

Caldariellaquinone {6-(3,7,11,15,19,23-hexamethyltetracosyl)-5-methylthiobenzo[*b*]thiophen-4,7-quinone (7)} has been isolated from cultures of *Caldariella acidophila*, an extremely thermophilic and acidophilic bacterium. The structure was determined by degradation, spectroscopic, and ^{13}C biosynthetic studies.

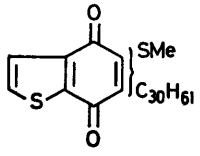
A RANGE of acidophilic, thermophilic bacteria, designated MT strains,[1] which resemble the species *Sulfolobus acidocaldarius*,[2] *Thermoplasma acidophila*,[3] and the pleiomorphic iron or sulphur autotroph of Brierly and Brierly,[4] have been isolated from hot acid springs at Pisciarelli near Naples. De Rosa *et al.*[5] propose that all these organisms be grouped together under the name *Caldariella*. All are acidophilic (pH optima 2.0—5.0) and very thermophilic (up to 89 °C in the MT series), and grow on simple media. They are also distinguished by the complete absence of ester lipids, and, more significantly, the structures of all the major lipids of these organisms are based upon a cyclic 1,2-diether of glycerol with bidentate C_{40} isoprenoid units [6] which are formed by head-to-head linkage of two *O*-phytanyl chains, and include further cyclic structures in the major component.[7] We now describe a unique terpenoid benzo[*b*]thiophen-4,7-quinone elaborated by *Caldariella acidophila*.[8]

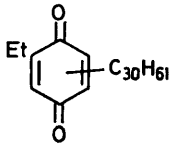
Caldariellaquinone was isolated from the total lipid extract of the lyophilised cells as an orange-red oil, $C_{39}H_{66}O_2S_2$, which showed $\lambda_{max.}$ (MeOH) 241, 283, 333, and 471 nm, and $\nu_{max.}$ 1 668 and 1 647 cm^{-1}, reminiscent of 1,4-naphthoquinones.[9] On reductive acetylation it formed a leucodiacetate, $\lambda_{max.}$ 227, 258, 297, and 308 nm. The n.m.r. spectrum of the quinone showed a two-proton AB quartet centred at δ 7.55 and 7.47 (*J* 5 Hz), and a methyl singlet at δ 2.62. The remainder of the spectrum corresponded to a C_{30} saturated isoprenoid chain showing a triplet at δ 2.75 (*J* 7 Hz) for quinone-CH_2·CH_2, a broad signal centred at 1.20 for methylene and methine protons, a three-proton doublet (*J* 6 Hz) at 0.98 from the methyl group of the first isoprene unit, and overlapping doublets at 0.87 and 0.85 for the remaining six methyl groups. The presence of this side chain was confirmed by oxidation with alkaline hydrogen peroxide followed by methylation with diazomethane. Analysis of the resulting mixture by g.l.c.–mass spectrometry showed, in addition to a series of minor components, a

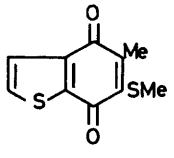
[1] M. De Rosa, A. Gambacorta, and J. D. Bu'Lock. *J. Gen. Microbiology*, 1975, **86**, 156; G. Millonig, M. De Rosa, A. Gambacorta, and J. D. Bu'Lock, *ibid.*, p. 165.
[2] T. D. Brock, K. M. Brock, T. R. Belly, and L. R. Weiss, *Arch. Microbiology*, 1972, **84**, 54.
[3] G. Darland, T. D. Brock, W. Samsonoff, and S. F. Conti, *Science*, 1970, **170**, 1416.
[4] C. L. Brierly and J. A. Brierly, *Canad. J. Microbiology*, 1973, **19**, 183.
[5] M. De Rosa, A. Gambacorta, G. Millonig, and J. D. Bu'Lock, *Experientia*, 1974, **30**, 866.
[6] M. De Rosa, A. Gambacorta, and J. D. Bu'Lock, *Phytochemistry*, 1976, **15**, 143.
[7] M. De Rosa, A. Gambacorta, L. Minale, and J. D. Bu'Lock, *J.C.S. Chem. Comm.*, 1974, 543.
[8] M. De Rosa, A. Gambacorta, and L. Minale, *J.C.S. Chem. Comm.*, 1975, 392.
[9] R. H. Thomson, 'Naturally Occurring Quinones,' 2nd edn., Academic Press, London, 1971, p. 39.

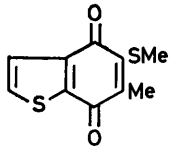
major product with M^+ 480 and important fragments of *m/e* 74(22%) [CH_2=C($\overset{+\cdot}{O}$H)·OCH_3] and 87(100%) [CH_2=CH·C(=$\overset{+}{O}$H)·OCH_3], consistent with the methyl ester of a saturated C_{31} acid.

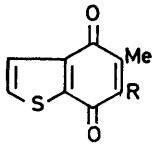
From this it appeared that the quinone contains a $C_{30}H_{61}$ chain linked to a $C_9H_5O_2S_2$ residue, which could be formulated as a benzo[*b*]thiophen-4,7-quinone with a methylthio-substituent as shown in (1), the spectroscopic data being in general agreement with those of synthetic benzo[*b*]thiophen-4,7-quinones.[10] For example, the parent quinone shows $\lambda_{max.}$ (MeOH) 255, 263sh, 315, 374sh, and 465 nm, $\nu_{max.}$ (film) 1 662 and 1 648 cm^{-1}, and an AB quartet centred at δ 7.61 and 7.53 arising from the thiophen ring protons. In agreement, desulphurisation with Raney nickel[11] led to an ethyl-(C_{30}-alkyl)-1,4-benzoquinone (2), M^+ 556, showing

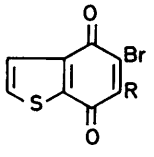

(1)


(2)


(3)


(4)


(5)


(6)

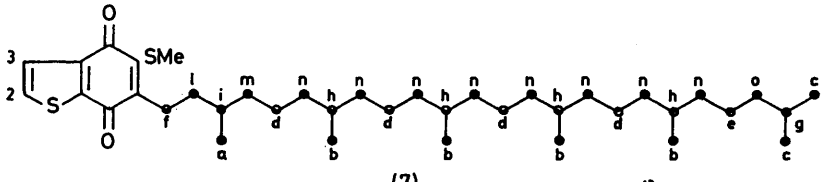
a signal for two quinonoid protons in the n.m.r. spectrum at δ 6.40, and a methyl triplet at 1.15 (*J* 6 Hz) which collapsed to a singlet on irradiation at δ 2.3 (quinone-CH_2 region).

Our next concern was to establish the relative positions of the substituents on the quinonoid ring in caldariellaquinone, and the exact nature of the C_{30} isoprenoid chain. The first problem was resolved by synthesis of the model compounds 5-methyl-6-methylthiobenzo[*b*]-thiophen-4,7-quinone (3) and 5-methylthio-6-methylbenzo[*b*]thiophen-4,7-quinone (4). As expected both isomers have very similar spectroscopic properties, but they can be distinguished by their u.v. spectra. The isomer (3) showed $\lambda_{max.}$ 239, 252, 338, and 458 nm, whereas the u.v. curve of the isomer (4), with maxima at 238, 274, 325, and 462 nm, was much closer to that of the natural quinone. In the n.m.r. spectra of both isomers the methylthio-protons resonated at δ 2.64, but a very small difference was observed in the aromatic region and the line positions of the AB quartet due to the thiophen ring protons in the spectrum of (4) are in better accord with the shift values for the natural compound.

The model compounds were prepared as follows. Chlorination of 5-methylbenzo[*b*]thiophen-4,7-quinone (5; R = H)[12] gave the 6-chloro-derivative (5; R = Cl) which yielded (3) on reaction with sodium methanethiolate. The isomer (4) was obtained from 5-bromo-4-hydroxybenzo[*b*]thiophen[13] by oxidation with Fremy's salt to give 5-bromobenzo[*b*]thiophen-4,7-quinone (6; R = H), followed by methylation with acetic acid–persulphate–silver ion[14] to yield (6; R = Me), and replacement of the bromine with sodium methanethiolate.

The structure of the hexamethyltetracosyl side chain in caldariellaquinone was deduced from ^{13}C n.m.r. studies on the metabolite at natural abundance and on ^{13}C-enriched samples produced by cultures of *Caldariella acidophila* fed with [1-^{13}C]- and [2-^{13}C]-acetate. In two separate experiments, growing cultures of *Caldariella acidophila* (MT3 strain) were incubated with [1-^{13}C]-acetate (90%) and [2-^{13}C]-acetate (90%), and the labelled quinone was isolated after 36 h. The proton-noise-decoupled natural abundance and enriched Fourier transform ^{13}C spectra were obtained for solutions in chloroform.

The assignments for the isoprenoid chain carbons were based on chemical shifts, multiplicities in the off-resonance spectra, comparison with polyisoprenoid compounds such as vitamin E,[15,16] and selective enrichment. The shift data, along with the peak heights, normalised with respect to that of the S·CH_3 signal, are reported in the Table, and the enriched carbon positions are indicated in formula (7). These data fully define the nature and the biogenetic origin of the C_{30} isoprenoid chain. They also exclude the possibility of a head-to-


(7)

• from [2-^{13}C] acetate
o from [1-^{13}C] acetate

[10] I. Baxter and B. A. Davis, *Quart. Rev.*, 1971, **25**, 239; C. J. P. Spruit, *Rec. Trav. chim.*, 1962, **81**, 810.
[11] F. F. Blick and D. G. Sheets, *J. Amer. Chem. Soc.*, 1949, **71**, 4010.
[12] D. S. Tarbell, D. K. Fukishima,and H. Dam, *J. Amer. Chem. Soc.*, 1945, **67**, 1643.
[13] E. Campaigne, A. Dinner, and M. Haseman, *J. Heterocyclic Chem.*, 1971, **8**, 755.
[14] N. Jacobsen and K. Torssell, *Annalen*, 1972, **763**, 135.
[15] Le Roy F. Johnson and W. C. Jankowski, 'Carbon-13 NMR Spectra; a Collection of Assigned, Coded, and Indexed Spectra,' Wiley–Interscience, New York, 1972.
[16] M. Matsuo and S. Urano, *Tetrahedron*, 1976, **32**, 229.

head linkage of prenyl units (as in the cyclic diether lipids from the same organism [7]) by the absence of a signal at δ_C *ca.* 12—13 from a terminal methyl carbon atom in a structure $CH_3{\cdot}CH_2{\cdot}CH(CH_3)-$. [A squalene type structure, *i.e.* two C_{15} units linked tail-to-tail, was eliminated by the ^1H n.m.r. spectrum, which showed the presence of a quinone–$CH_2{\cdot}CH_2{\cdot}CH(CH_3)-$ structure.]

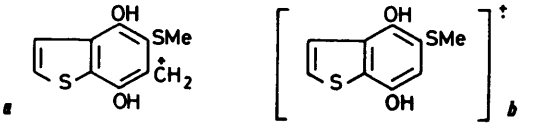
Incorporation of [1-^{13}C]acetate (○) and [2-^{13}C]acetate (●) into caldariellaquinone (7) [a]

δ_C(CDCl$_3$)	Multiplicity	Assignment	Peak height [b] Natural sample	[1-^{13}C]-acetate-enriched sample	[2-^{13}C]-acetate-enriched sample
17.91	q	SMe	1.00	1.00	1.00
19.53	q	a	1.92	1.55	●3.45
19.78	q	b	5.44	6.55	●9.72
22.67	q	c	1.72	1.70	●2.90
24.45	t	d	5.32	○17.4	5.40
24.77	t	e	2.00	○4.00	2.02
26.66	t	f	1.65	○4.00	1.80
27.95	d	g	1.02	○1.93	0.93
29.68 [c]					
32.77	d	h	8.01	○14.9	7.02
33.35	d	i	1.84	○4.38	1.60
35.60	t	l	1.56	1.51	●3.78
36.94	t	m	2.00	2.31	●6.04
37.41	t	n	10.4	13.02	●28.78
39.37	t	o	1.54	1.00	●2.50
126.35	d	C-2 or C-3	1.53		
132.39	d	C-3 or C-2	1.0ε		
141.58	s	sp^2 quaternary	0.44		
142.08	s		0.20		
145.41	s		0.44		
149.84	s		0.46		
176.10		carbonyl	0.1		
176.16			0.1		

[a] ^{13}C N.m.r. spectra were determined at 25.20 MHz with an XL-100 Varian Fourier transform spectrometer, operating in both proton-noise decoupled and off-resonance decoupled modes. Me_4Si was used as internal standard. [b] Normalised with respect to SMe signal. [c] Probably impurity.

The quartet at δ_C 17.91 in the ^{13}C spectrum was assigned to S·*C*H$_3$, which did not show enrichment from either [1-^{13}C]- or from [2-^{13}C]-acetate (thioanisole is reported to exhibit a methyl carbon signal at δ_C 15.6 [14]). The resonances at δ_C 132.39 and 126.35, which appeared as doublets in the off-resonance spectrum, were attributed to C-2 and -3, the off-resonance singlets associated with the quaternary carbon atoms of the quinonoid ring were found at δ_C 141.58, 142.08, 145.41, and 149.84, and the carbonyl carbon signals were at δ_C 176.10 and 176.16. None of these ring carbon atoms showed enrichment in the labelled quinone samples.

Further support for structure (7) comes from the mass spectrum, which is dominated by fragmentation of the polyprenyl side chain, a major peak appearing at *m/e* 225 ($C_{10}H_9O_2S_2$ by accurate mass measurement). We ascribe this to ion *a*, reduction of the quinone occurring either by adventitious water or by hydrogen transfer from the side chain. Complete loss of the side chain also occurs to give the ion *b*, *m/e* 212.



Caldariellaquinone is structurally analogous to the polyprenylnaphthoquinones (menaquinones) found in many bacteria,[17] which suggests a possible respiratory function [18] for it in *Caldariella acidophila* which is a sulphur autotroph. This view is supported by the absence of both menaquinones and ubiquinones (as judged by chromatography against authentic samples.) The co-existence of this unique quinone and the unique cyclodiether lipids is presumably related to the unusual environment (pH 1.4—2.6; 74—89 °C) from which the organism was isolated, and the corresponding peculiarities of membrane structure which such conditions may well require. It now seems particularly desirable to establish the presence of this quinone in the other *Caldariella* organisms, all of which possess cyclodiether lipids.[6]

EXPERIMENTAL

G.l.c.–mass spectrometry measurements were carried out with an A.E.I. MS30 instrument connected to a Pye gas chromatograph equipped with a 1.5 m × 1.5 mm (i.d.) glass column packed with SE-30 3% on GasChrom Q (100—120 mesh) (temp. 280 °C).

Organism and Culture Conditions.—An isolate of *Caldariella acidophila* (MT3 strain),[1] obtained from hot springs in the volcanic area of Naples, was used. The medium contained (g l^{-1}): yeast extract (Difco), 1.0; casamino acids (Difco), 1.0; KH_2PO_4, 3.1; $(NH_4)_2SO_4$, 2.5; $MgSO_4{,}7H_2O$, 0.20; $MgCl_2{,}2H_2O$, 0.25; tap water to 1 l; pH adjusted with H_2SO_4. The organism was grown in 25 l batches at 73 °C with aeration (2.5—3.0 l min^{-1}) and agitation in a 30 l fermentor (Terzano). The culture vessel was inoculated by adding 3 l of an 18 h broth culture. The labelled substrates, sodium [1-^{13}C]acetate (500 mg; 90%) and sodium [2-^{13}C]-acetate (500 mg; 90%) (Merck, Sharp, and Dohme), dissolved in water (100 ml), were added to the cultures at the beginning of the exponential phase over 3 h. Cells were harvested in the stationary growth phase (36 h incubation) by continuous-flow centrifugation, washed with 0.1M-NaCl, and freeze-dried (yield 14—16 g of dried cells from 25 l of culture).

Extraction of Lipids and Isolation of Caldariellaquinone (7).—The dried cells (200 g) were extracted (Soxhlet) for 48 h with chloroform–methanol (1 : 1 v/v). After evaporation the total lipid residue was treated with light petroleum (b.p. 40—70 °C), and the soluble fraction was chromatographed on a silica gel column in light petroleum containing increasing amounts of ether. The *quinone* was recovered from the 5% ether fractions as a viscous orange oil (110 mg) (0.055% dry weight of cells) (Found: S, 9.5%; M^+, 630.4521. $C_{39}H_{66}O_2S_2$ requires S, 10.1%; *M*, 630.4504); $[\alpha]_D$ −5° (*c* 1 in $CHCl_3$); $\lambda_{max.}$ (MeOH) 241, 283, 333, and 471 nm (log ε 4.11, 3.90, 3.70, and 3.07); $\nu_{max.}$ (film) 1 668 and 1 647 cm^{-1}; *m/e* 630(4.5%), 598.4784(1.5) ($C_{39}H_{66}O_2S$ requires 598.4782), 583(1.0), 225.0048(14) ($C_{10}H_9O_2S_2$

[17] D. R. Threlfall and G. R. Whistance, in 'Aspects of Terpenoid Chemistry and Biochemistry,' ed. T. W. Goodwin, Academic Press, London, 1971.

[18] R. Bentley and I. M. Campbell in ' The Chemistry of the Quinonoid Compounds,' ed. S. Patai, Wiley, London, 1974.

requires 225.0041), 223.9964(11) ($C_{10}H_8O_2S_2$ requires 223.9965), 211.9963(45) ($C_9H_8O_2S_2$ requires 211.9965), 192(2), 179(3.5), 85(72), and 83(100); for n.m.r. data see Discussion section.

Reactions of Caldariellaquinone.—(a) *Reductive acetylation.* The quinone (20 mg) in methanol (1 ml) and ether (0.5 ml) was treated with an excess of sodium borohydride. The decolourised solution was evaporated under nitrogen, and acetic anhydride (1 ml) and pyridine (2 drops) were added rapidly. Next day the mixture was taken to dryness *in vacuo*, and the residue was purified by t.l.c. on silica gel in light petroleum (b.p. 40—70 °C)–ether (85 : 15) to give the leucodiacetate as an oil (10 mg) (R_F 0.45; intense blue u.v. fluorescence) (Found: M^+, 716. $C_{43}H_{72}O_4S_2$ requires M, 716); $\lambda_{max.}$ (MeOH) 227, 258, 297, and 308 nm (log ε 4.30, 4.05, 3.29, and 3.31), $\nu_{max.}$ (film) 1 770 cm^{-1}, δ (CCl_4) 7.10 and 7.28 (each 1 H, d, *J* 5 Hz, H-2 and -3), 2.82 (2 H, t, *J* 6 Hz, $ArCH_2{\cdot}CH_2$), 2.28, 2.33, and 2.37 (each 3 H, s, SMe and $MeCO_2$), 1.00 (3 H, d, *J* 5.5 Hz, $ArCH_2{\cdot}CH_2{\cdot}CHMe$), and 0.88 and 0.85 (18 H, overlapped doublets, *J* 6 Hz, the other $>CHMe$); *m/e* 716(0.7%), 674(3.5), and 632(100), 617(1), 600(3.5), 586(6), 267(5), 225(32), and 179(18).

(b) *Degradation with alkaline hydrogen peroxide.* The quinone (10 mg) in methanol (1 ml) and 2M-sodium hydroxide (1 ml) was added to 30% hydrogen peroxide (100 vol; 0.5 ml). The solution was heated on a steam-bath for 30 min with further addition of peroxide (4.5 ml). The cooled mixture was acidified with 5M-hydrochloric acid and extracted with ether. The extract was washed, dried ($MgSO_4$), and treated with an excess of diazomethane. After removal of the solvent the crude ester was purified by t.l.c. on silica gel in light petroleum (b.p. 40—70 °C)–ether (95 : 5). The major band (R_F 0.70) yielded an oily residue (4 mg) which on g.l.c.–mass spectrometry gave a single peak (t_R 12.4 min) [*m/e* 480 (M^+, 1.5%), 423(10), 353(2), 157(18), 87(100), and 74(22)].

Ubiquinone-6, after hydrogenation, was oxidised in a similar manner and gave a methyl ester with identical behaviour in g.l.c.–mass spectrometry.

(c) *Degradation with Raney nickel.* The quinone (48.5 mg) in methanol (8 ml) was refluxed with Raney nickel W-7 [11] (1 g) for 3 h. The cooled mixture was filtered, and the filtrate was taken to dryness. The residue, which turned yellow, was chromatographed on a column of silica gel (10 g) with light petroleum (b.p. 40—70 °C) containing increasing amounts of ether as eluant. The 2% ether fraction yielded the dialkylquinone (2) as a pale yellow oil, R_F 0.8 on silica in light petroleum–ether (95 : 5); $\lambda_{max.}$ (MeOH) 257, 320, and 436 nm (log ε 3.97, 2.91, and 2.01); $\nu_{max.}$ (film) 1 655 and 1 610 cm^{-1}; *m/e* 558(3%), 556(M^+, 0.5), 312(9), 269(8), 267(7), 151(13), 101(71), and 88(100); δ (CCl_4) 6.40br (2 H, s, –CH=), 2.30 (4 H, m, quinone–CH_2), 1.15 (3 H, t, *J* 6 Hz, quinone–$CH_2{\cdot}CH_3$), 1.1 (38 H, m, CH_2 and CH), 0.95 (3 H, d, *J* 6 Hz, 3′-Me), and 0.88 and 0.85 (18 H, overlapping d, the other $CHMe$-groups).

6-Chloro-5-methylbenzo[b]*thiophen-4,7-quinone* (5; R = Cl).—A saturated solution of chlorine in acetic acid (1 ml) was added to 5-methylbenzo[*b*]thiophen-4,7-quinone [12] (94 mg) in the same solvent (1 ml), and the mixture was stirred for 30 min. After removal of the excess of chlorine *in vacuo*, anhydrous sodium acetate (59 mg) was added, and the mixture was boiled for 10 min. Water was then added, dropwise, to effect complete dissolution at the boil. On cooling, the *chloroquinone* separated as pale yellow needles, m.p. 148.5—149° (from ethanol) (62 mg) (Found: S, 15.2%; M^+, 211.9700. $C_9H_5{}^{35}ClO_2S$ requires S, 15.1%; *M*, 211.9698); $\lambda_{max.}$ (EtOH) 226, 276sh, 283, 338, and 390sh nm (log ε 4.02, 4.14, 4.20, 3.49, and 3.04); $\nu_{max.}$ (KBr) 1 668, 1 636sh, 777, 747, and 722 cm^{-1}; δ ($CDCl_3$) 7.71 and 7.55 (each 1 H, d, *J* 5 Hz, H-2 and -3) and 2.28 (3 H, s, Me); *m/e* 214(28%), 213(5), 212(100), 184(10), 177(45), 149(71), 121(11), 110(20), 84(6), and 77(6).

5-Methyl-6-methylthiobenzo[b]*thiophen-4,7-quinone* (3).—To a stirred solution of the above chloroquinone (62 mg) in benzene (7 ml) sodium methanethiolate (25 mg) in methanol (1 ml) was added, dropwise. After 3 h the solvents were removed *in vacuo*, and the residue was chromatographed on silica gel plates in ether–light petroleum (b.p. 60—80 °C) (1 : 3). After three elutions the contiguous yellow and orange bands were removed and re-run in benzene. The orange band yielded *5-methyl-6-methylthiobenzo*[b]*thiophen-4,7-quinone* as red needles, m.p. 83° (from methanol) (23 mg) (Found: M^+, 223.9966. $C_{10}H_8O_2S_2$ requires *M*, 223.9965); $\lambda_{max.}$ (MeOH) 239, 252, 270sh, 338, and 458 nm (log ε 3.95, 4.01, 3.82, 3.64, and 2.86); $\nu_{max.}$ (KBr) 1 650, 1 630, 770, 737, and 718 cm^{-1}; δ ($CDCl_3$) 7.62 and 7.49 (each 1 H, d, *J* 5 Hz, H-2 and -3), 2.64 (3 H, s, SMe), and 2.26 (3 H, s, CMe); *m/e* 225(6%) 224(100), 223(8), 209(15), 191(12), 181(5), 163(6.5), and 110(13).

5-Bromobenzo[b]*thiophen-4,7-quinone* (6; R = H).—To 5-bromo-4-hydroxybenzo[*b*]thiophen (0.87 g) in acetone (95 ml) was added Fremy's salt (2.74 g) in water (190 ml) containing potassium dihydrogen phosphate (0.87 g), during 10 min. The *quinone* was collected next day and recrystallised from ethanol to yield pale yellow-brown needles, m.p. 131.5° (0.5 g) (Found: S, 13.1%; M^+, 241.9038. $C_8H_3{}^{79}BrO_2S$ requires S, 13.2%; *M*, 241.9037); $\lambda_{max.}$ (EtOH) 227, 287, 325, and 380sh nm (log ε 3.99, 4.04, 3.33, and 3.15); $\nu_{max.}$ (KBr) 1 670, 1 642, 740, 734, and 690 cm^{-1}; δ ($CDCl_3$) 7.70 and 7.62 (each 1 H, d, *J* 5 Hz, H-2 and -3) and 7.39 (1 H, s, H-6), *m/e* 244(50%), 242(47), 163(30), 136(12), 135 (100), 110(21), 84(10), 82(6.5), and 81(8).

5-Bromo-6-methylbenzo[b]*thiophen-4,7-quinone* (6; R = Me).—To 5-bromobenzo[*b*]thiophen-4,7-quinone (377 mg) in acetonitrile (9 ml) containing acetic acid (0.13 ml), water (5 ml), and silver nitrate (190 mg), stirred at 60—65 °C, was added ammonium persulphate (710 mg) in water (3.5 ml) during 1 h. After stirring for a further 10 min at 60—65 °C the mixture was cooled to room temperature, neutralised with sodium hydrogen carbonate, extracted with ether, and evaporated. The residue was passed down a column of silica gel (thrice) in benzene, and then crystallised from ethanol to give the *bromomethylquinone* as pale yellow-brown needles, decomp. 157—161° (151 mg) (Found: S, 12.3%; M^+, 255.9187. $C_9H_5{}^{79}BrO_2S$ requires S, 12.5%; *M*, 255.9192); $\lambda_{max.}$ (EtOH) 226, 290, 341, and 329sh (log ε 4.02, 4.15, 3.43, and 3.09); $\nu_{max.}$ (KBr) 1 665, 1 645, 792, 746, and 708 cm^{-1}; δ ($CDCl_3$) 7.67 and 7.58 (each 1 H, d *J* 5 Hz, H-2 and -3), and 2.34 (3 H, s, Me); *m/e* 258(47%), 256(50), 177(63), 149(100), 121(23), and 110(18).

6-Methyl-5-methylthiobenzo[b]*thiophen-4,7-quinone* (4).—A solution of sodium methanethiolate (35 mg) in methanol (1 ml) was added to 5-bromo-6-methylbenzo[*b*]thiophen-4,7-quinone (113 mg) in benzene (10 ml). The solution was kept at 50 °C for 1 h and the solvents were then removed *in vacuo*. The residual mixture was purified by p.l.c. on silica gel and the major orange product was crystallised from methanol to give the *quinone* (4) as red needles, m.p. 116—117° (26 mg) (Found: M^+, 223.9964. $C_{10}H_8O_2S_2$ requires *M*, 223.9965); $\lambda_{max.}$ (EtOH) 238, 274, 325, and 462 nm

(log ε 4.05, 3.79, 3.69, and 3.12); $\nu_{max.}$ (KBr) 1 662, 1 626, 810, 757, and 717 cm^{-1}; δ ($CDCl_3$) 7.61 and 7.51 (each 1 H, d, *J* 5 Hz, H-2 and -3), 2.65 (3 H, s, SMe), and 2.28 (3 H, s, CMe), *m/e* 225(6.5%), 224(100), 223(7.5), 209(17), 191(10), 181(5), 163(6), 149(7.5), and 110(17).

We thank Dr. R. M. Scrowston for a gift of benzo[*b*]-thiophen-4-yl methylcarbamate, Mr. C. Di Pinto for ^{13}C n.m.r. spectra, and Messrs. E. Esposito and S. Sodano for technical assistance.

[6/1962 *Received*, 21*st October*, 1976]