Tetrahedron Vol 43, No 1, pp 263 to 268, 1987
Printed in Great Britain.

0040-4020/87 $5.00+.00

ANTITUMOR CYCLIC PEROXIDES FROM THE SPONGE *PLAKORTIS LITA*

SHINICHI SAKEMI and TATSUO HIGA*

Harbor Branch Oceanographic Institution, SeaPharm Research Laboratories, 5600 Old Dixie Highway, Fort Pierce, Florida 33450, USA
and
Department of Marine Sciences, University of the Ryukyus, Nishihara, Okinawa 903-01, Japan

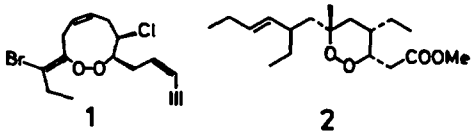
UFFE ANTHONI and CARSTEN CHRISTOPHERSEN*

Marine Chemistry Section, Department of General and Organic Chemistry, University of Copenhagen, The H.C. Ørsted Institute, DK-2100 Copenhagen, Denmark

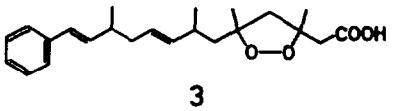
(*Received in UK* 10 *November* 1986)

Abstract: Four new cyclic peroxides (**5**-**8**) along with known chondrillin (**4**) have been isolated as antitumor constituents of the sponge *Plakortis lita*. These are methyl-(3*S**,6*S**)-3,6-epidioxy-6-methoxyoctadec-4-enoate (**5**), methyl(3*S**,6*S**,16*E*)-3,6-epidoxy-6-methoxyoctadeca-4,16-dienoate (**6**), methyl(3*S**,6*S**,14*E*,16*E*)-3,6-epidioxy-6-methoxyoctadeca-4,14,16-trienoate (**7**), and methyl-(3*S**,6*S**,16*E*,18*E*)-3,6-epidioxy-6-methoxyeicosa-4,16,18-trienoate (**8**). All of them are epimeric to **4** and exhibit higher antitumor potency than **4**.

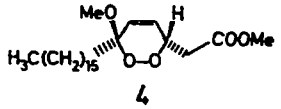
Other than steroidal peroxides,[1] about 25 cyclic peroxides have hitherto been reported from marine organisms. With a few exceptions, *e.g.* rhodophytin (**1**) from *Laurencia*,[2] most are sponge metabolites and carboxylic acid derivatives of terpenoids[3-6] as well as non-terpenoids.[7-11] The latter type peroxides are mainly composed of branched chain carboxylic acids such as plakortin (**2**)[8] and plakinic acid A (**3**).[11] Chondrillin (**4**)[7] has been the only known example derived from a straight-chain fatty acid. Biological activity of these compounds encompasses antimicrobial activity,[6,8-12] ichthyotoxicity,[4] and cytotoxicity.[5,11]



1 **2**



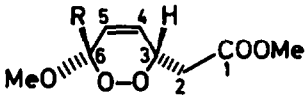
3



4

In our screening for biologically active metabolites from marine organisms from Okinawan waters, an extract of the sponge *Plakortis lita* de Laubenfels, 1954, exhibited significant antitumor activity against P388 mouse leukemia cells. Separation of the extract gave five active constituents including chondrillin (**4**). This paper describes the isolation and structures of these compounds.

A freeze-dried sample of the sponge *P. lita* was extracted with ethyl acetate to give an oil. The extract was repeatedly chromatographed on silica gel, and a P388 active fraction was subjected to purification using countercurrent chromatography which served to separate minor active constituents from the major product, chondrillin (4). Further purification of these fractions by HPLC gave five pure cyclic peroxides 4-8. The major product which amounted to more than 95% of the total yield of the isolated compounds was identified as the known peroxide chondrillin (4) by comparison of spectral data. Chondrillin (4) was first isolated by Wells from the sponge *Chondrilla* sp.[7]



5 R = $-(CH_2)_{11}\overset{18}{C}H_3$

6 R = $-(CH_2)_9\overset{16}{C}H=\overset{17}{C}H\overset{18}{C}H_3$

7 R = $-(CH_2)_7\overset{14}{C}H=CH-CH=\overset{18}{C}H_3$

8 R = $-(CH_2)_9\overset{16}{C}H=CH-CH=\overset{20}{C}H_3$

Spectral data indicated that the four minor compounds (5-8) were closely related to **4**. The presence of a methyl ester function in all of them is shown by the same IR absorption band at 1735 cm^{-1} and identical ^1H NMR signal at δ 3.72 (3 H, s, OCH_3). As shown in Table 1 all the compounds 5-8 exhibited virtually

Table 1. Selected ^1H NMR chemical shifts for Peroxides 4-8.

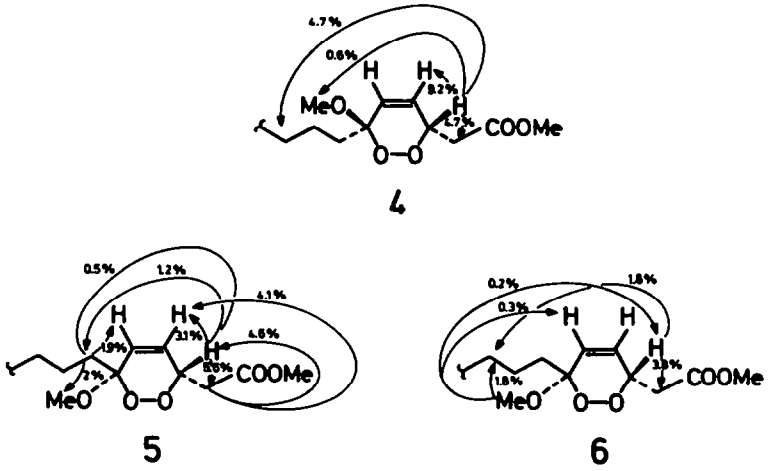
Proton	4	5	6	7	8
2-H	2.26	2.52	2.52	2.51	2.51
	2.93	2.62	2.62	2.62	2.61
3-H	4.78	5.01	5.02	5.01	5.00
4-H	6.18	6.12	6.12	6.12	6.11
5-H	5.85	5.86	5.85	5.85	5.84
C_6-OCH_3	3.40	3.39	3.40	3.39	3.38
$COOCH_3$	3.73	3.72	3.73	3.72	3.72
CH_3	0.88	0.87	1.64	1.73	1.72

identical ^1H NMR signals for H-2, H-3, H-4, H-5 and the two methoxy groups, suggesting the same stereostructure for the epidioxy ring portion. The signals for H-2, H-3, and H-4 were slightly but distinguishably different from those of **4**, indicating a stereochemical difference in the ring between **4** and 5-8. This was further substantiated by ^{13}C NMR data for the ring carbons which were identical for 6-8 [(δ 73.5 (C-3), 127.0 (C-4 or C-5), 130.4 (C-5 or C-4), 101.1 C-6)] and slightly different for **4** (δ 73.7, 126.4, 129.2, 100.5). The difference among the compounds 5-8 lies in the side chain structures. All of them contain a straight

chain as revealed by the presence of only one terminal methyl group (5: δ 0.87t; 6: δ 1.64d; 7: δ 1.73d; 8: δ 1.72d) and a broad signal at δ 1.25 for methylene protons. Like chondrillin (4) EIMS of all the new compounds (5-8) showed a characteristic M - O_2 peak as the first fragment ion. Compound 5 exhibited this ion at m/z 324 ($C_{20}H_{36}O_3$). Thus, the molecular formula of 5 is $C_{20}H_{36}O_5$, and 5 contains a saturated side chain ($C_{12}H_{25}$). The M - O_2 peak of 6 was observed at m/z 322 ($C_{20}H_{34}O_3$), indicating the presence of an additional unsaturation site. This together with the olefinic signals in the ^1H [δ 5.42 (2 H, m)] and ^{13}C NMR spectra (δ 124.6d, 131.7d) revealed the presence of a double bond in the side chain. The double bond was located next to the terminal carbon which was observed as a vinyl methyl doublet (δ 1.64). The *E*-configuration was assigned for the double bond owing to a strong IR absorption band at 958 cm^{-1} and a quartet at δ 17.9 in the ^{13}C NMR spectrum.[13]

The EIMS of 7 had the M-O_2 peak at m/z 320 ($C_{20}H_{32}O_3$), suggesting the presence of two sites of unsaturation in the side chain. The ^1H [δ 5.56 (2H, m), 6.00 (2 H, m)] and ^{13}C NMR data (δ 126.7d, 130.3d, 131.7d, 132.1d) confirmed them to be due to two double bonds. The first double bond was placed on the carbon next to the terminal methyl group which appeared as a vinyl doublet at δ 1.73. The fact that this signal is slightly deshielded comparing to that of 6 and the absence of no more than one allylic methylene signal [δ 2.03 (2 H, m)] are the evidence for the second double bond to be in conjugation with the first double bond. A shift of IR absorption band to 981 cm^{-1} from 958 cm^{-1} of 6 suggested the *E,E*-configuration for both double bonds in 7.

The ^1H NMR spectrum of 8 was almost identical with that of 7, except for the intensity of the methylene signal of 8, suggesting that 8 is a higher homologue of 7. The M - O_2 peak at m/z 348 in the EIMS indicated that the side chain of 8 had an additional two methylene units compared to 7.



4

5

6

Figure 1.

Wells reported that the absolute configuration of chondrillin (4) at C-3 as *S* based on CD and ORD studies on a 3-hydroxy derivative prepared by hydrogenation of 4. The configuration at C-6 has remained unknown, however. In order to establish the relative configurations of these compounds, we carried out NOE studies on 4, 5, and 6. In the NOE difference spectrum of 4, irradiation of the signal at δ 4.78 (H-3) caused the disappearance of the signals for H-5 (δ 5.85)

and H-7 (δ 1.63) and enhancement of the signals for H-4 (δ 6.18, 9.2%), H-2 (δ 2.62 and 2.93, 4.7%), C_6-OMe (δ 3.40, 0.6%), and $-CH_2-$ (δ 1.25, 4.7%) (Figure 1). Although the effect is not large, the result suggested that the proton on C-3 and the methoxy group on C-6 are oriented *cis* in **4**. As shown in Figure 1, the results with **5** and **6** are consistent with the conclusion that they have configurations epimeric to **4**. These stereochemical assignments are in agreement with the result of Crews[14] who assigned the relative configurations of homologous cyclic peroxides on the basis of conformational analysis and NMR coupling constant arguments.

Compound **4** showed IC_{50} 5 μg/ml in *in vitro* antitumor assay against P388 cells, while the epimers **5-8** exhibited IC_{50} 0.05-0.1 μg/ml. These results demonstrated the importance of the epidioxy ring stereochemistry for enhancement of the activity.

It is curious that chondrillin (**4**) is a major metabolite in *Plakortis lita* de Laubenfels[15,16] of order Homosclerophorida, family Plakinidae as well as *Chondrilla* sp.[7] of order Hadromerida, family Chondrosiidae since these two sponges are not at all related. Furthermore another plakinid sponge, *Plakortis halichondroides* (Wilson) yielded plakortin derived from a branched chain C_{17} precursor[8] while a chondrosiid sponge, *Chondrosia collectrix* have branched chain cyclic peroxides.[9] Two epimeric peroxides, xestin A and B, homologous to **7** and **8** were isolated from *Xestospongia* sp.[14] of still another order and family (Nepheliospongia, Nepheliospongiidae). Apparently the type of peroxide has no taxonomic significance. As to the biogenesis of these compounds nothing is known at present, however, some sponges e.g. *Iotrochota birotulata* have peroxidases.[17,18]

EXPERIMENTAL

Infrared spectra were measured on a Perkin Elmer 1310 Infrared spectrophotometer and optical rotations on an Optical Activity AA-5 digital polarimeter. ^1H and ^{13}C NMR spectra were taken on a Bruker AM 360 and NOE difference spectra on a Bruker AM 500 spectrometer. Mass spectra were recorded at Mass Spectrometry Center, University of Illinois at Urbana-Champaign.

Extraction and Isolation

A sample of the sponge *Plakortis lita* (416 g dry weight), collected at Onna, Okinawa, was freeze-dried and extracted with ethyl acetate to give 20 g of oil. The oil was separated on a silica gel column by eluting with 5:1 heptane-ethyl acetate into four fractions. The second fraction (3.8 g) was again loaded on the same column and eluted with the same solvent system to furnish four subfractions. The most active subfraction on the P388 assay was subjected to the separation by countercurrent chromatography[19] using 5:1:4 heptane-methylene chloride-acetonitrile as solvent (mobile phase: upper layer). Each of the active mobile phase fractions was repeatedly separated by HPLC on Altex ODS (CH_3CN-H_2O) and/or LiChrosorb Si-60 column (heptane-EtOAc) to yield the peroxides **4** (800 mg), **5** (14 mg), **6** (7 mg), **7** (10 mg), and **8** (4 mg).

Chondrillin (4)

Compound **4** was obtained as a white solid, mp 31.5°C; $[\alpha]_D^{20}$ +40.0° (c 4.5, MeOH); IR (KBr) 2942, 2908, 2840, 1735, 1462, 1434, 1247, 1160, 1142, 1126, and 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (3 H, t, J = 6.7 Hz), 1.25 (br s), 1.63 (2 H,

m), 2.62 (1 H, dd, J = 5.2, 16.1 Hz), 2.93 (1 H, dd, J = 8.1, 16.1 Hz), 3.40 (3 H, s), 3.73 (3 H, s), 4.78 (1 H, m), 5.85 (1 H, dd, J = 1.8, 10.3 Hz), and 6.18 (1 H, dd, J = 4.3, 10.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.07 (q), 22.65 (t), 23.45 (t), 29,34 (t), 29.52 (t), 29.65 (t, 9 C), 31.90 (t), 34.29 (t), 37.18 (t), 50.96 (q), 51.90 (q), 73.66 (d), 100.45 (s), 126.43 (d), 129.20 (d), and 170.83 (s); HR EIMS m/z 380.3292 ($C_{24}H_{44}O_3$ requires 380.3291); LR EIMS m/z 380 (M - O_2, 69), 321 (28), 293 (10), 267 (10), 211 (9), 183 (6), 170 (10), 155 (9), 123 (27), 113 (88), 111 (29), 109 (21), 97 (45), 95 (22), 85 (23), 83 (27), 81 (19), 71 (42), 69 (37), 57 (89), 55 (61), 43 (100), 31 (25), and 30 (35 rel.%).

Methyl (3*S**,6*S**)-3,6-epidioxy-6-methoxyoctadec-4-enoate (5)

White solid, mp 49°C; $[\alpha]_D^{20}$ +38.1° (c 5.3, MeOH); IR (film) 2950, 2918, 2848, 1735, 1457, 1437, 1395, 1375, 1275, 1193, 1180, 1160, 1145, and 1068 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 6.7 Hz), 1.25 (br s), 1.66 (2 H, m), 2.52 (1 H, dd, J = 6.5, 16.1 Hz), 2.62 (1 H, dd, J = 7.5, 16.1 Hz), 3.39 (3 H, s), 3.72 (3 H, s), 5.01 (1 H, m), 5.86 (1 H, dd, J = 1.9, 10.0 Hz), and 6.12 (1 H, br d, J = 10.3 Hz); LR EIMS m/z 324 (M - O_2, 66), 265 (29), 237 (7), 211 (5), 183 (9.5), 170 (5), 155 (6), 153 (5), 123 (26), 113 (90), 111 (21), 109 (20), 97 (28), 95 (26), 83 (16), 81 (17), 71 (18), 69 (27), 67 (18), 59 (30), 57 (56), 55 (62), 43 (100), and 41 (82 rel.%).

Methyl (3*S**,6*S**,16*E*)-3,6-epidioxy-6-methoxyoctadeca-4,16-dienoate (6)

White solid, mp 37.5°C; $[\alpha]_D^{20}$ +41.4° (c 2.9, MeOH); IR (film) 2950, 2910, 2880, 2845, 1735, 1458, 1435, 1390, 1375, 1275, 1225, 1190, 1067, 985, and 958 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (br s), 1.32 (4 H, m), 1.64 (3 H, d, J = 6.4 Hz), 1.96 (2 H, m), 2.52 (1 H, dd, J = 6.6, 16.0 Hz), 2.62 (1 H, dd, J = 7.5, 16.0 Hz), 3.40 (3 H, s), 3.73 (3 H, s), 5.02 (1 H, m), 5.42 (2 H, m), 5.85 (1 H, dd, J = 2.2, 10.2 Hz), and 6.12 (1 H, dd, J = 1.4, 10.2 Hz); ^{13}C NMR ($CDCl_3$) δ 17.90 (q), 23.29 (t), 29.18 (t), 29.43 (t, 3 C), 29.63 (t), 29.76 (t), 32.61 (t), 34.84 (t), 36.38 (t), 51.30 (q), 52.06 (q), 73.51 (d), 101.11 (s), 124.55 (d), 126.97 (d), 130.40 (d), 131.69 (d), and 170.00 (s); LR EIMS m/z 322 (M - O_2, 31) 263 (13), 235 (5), 183 (8), 153 (4), 123 (13), 113 (48), 111 (15), 109 (17), 97 (23), 95 (20), 83 (13), 81 (22), 79 (11), 69 (34), 67 (25), 59 (22), 55 (100), 43 (24), and 41 (70 rel.%).

Methyl (3*S**,6*S**,14*E*,16*E*)-3,6-epidioxy-6-methoxyoctadeca-4,14,16-trienoate (7)

White solid, mp 38-39°C; $[\alpha]_D^{20}$ +40.8° (c 4.9, $CHCl_3$); IR (film) 3015, 2923, 2850, 1738, 1433, 1355, 1270, 1188, 1164, 1132, 1062, and 981 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 1.26 (6 H, br s), 1.34 (4 H, m), 1.65 (2 H, m), 1.73 (3 H, d, J = 6.5 Hz), 2.03 (2 H, m), 2.51 (1 H, dd, J = 6.5, 16.1 Hz), 2.62 (1 H, dd, J = 7.5, 16.2 Hz), 3.39 (3 H, s), 3.72 (3 H, s), 5.01 (1 H, dddd, J = 1.5, 2.2, 6.5, 7.5 Hz), 5.56 (2 H, m), 5.85 (1 H, dd, J = 2.2, 10.2 Hz), 6.00 (2 H, m), and 6.12 (1 H, dd, J = 1.5, 10.2 Hz); ^{13}C NMR ($CDCl_3$) δ 17.96 (q), 23.23 (t), 29.02 (t), 29.36 (t), 29.66 (t), 32.49 (t), 34.81 (t), 36.34 (t), 51.29 (q), 50.03 (q), 73.48 (d), 101.08 (s), 126.67 (d), 126.93 (d), 130.25 (d), 130.39 (d), 131.71 (d), 132.08 (d), and 170.00 (s); HR EIMS m/z 320.2352 ($C_{20}H_{32}O_3$ requires 320.2351); LR EIMS m/z 320 (M - O_2, 4) 304 (4), 270 (3), 201 (2.5), 193 (2.5), 187 (3), 183 (2.5), 179 (4), 166 (4.5), 156 (5), 153 (10), 147 (6), 135 (8), 133 (8), 125 (12), 124 (9.5), 123 (9.5), 121 (12.5), 113 (16), 107 (22), 96 (34), 95 (30), 94 (26), 93 (26), 91 (17), 83 (48), 81 (100), 79 (64), 77 (18), 68 (43), 67 (44), 59 (17), 55 (43), 53 (29), 43 (25), 41 (67), and 39 (25 rel.%).

Methyl (3*S**,6*S**,16*E*,18*E*)-3,6-epidioxy-6-methoxyeicosa-4,16,18-trienoate (8)

White solid, mp 47.5°C; $[\alpha]_D^{20}$ +36.4° (c 1.1, MeOH); IR (film) 3020, 2952, 2915, 2880, 2847, 1736, 1464, 1436, 1393, 1376, 1278, 1226, 1194, 1176, 1163, 1144, 1069, 978, and 955 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (br s), 1.33 (m), 1.64 (m), 1.72 (3 H, d, J = 6.4 Hz), 2.03 (2 H, m), 2.51 (1 H, dd, J = 6.6, 16.0 Hz), 2.61 (1 H, dd, J = 7.5, 16.0 Hz), 3.38 (3 H, s), 3.72 (3 H, s), 5.00 (1 H, dddd, J = 1.5, 2.1, 6.6, 7.5 Hz), 5.55 (2 H, m), 5.84 (1 H, dd, J = 2.1, 10.3 Hz), 5.99 (2 H, m), and 6.11 (1 H, dd, J = 1.5, 10.3 Hz); ^{13}C NMR ($CDCl_3$) δ 17.95 (q), 23.24 (t), 29.14 (t), 29.42 (t, 4 C), 29.71 (t), 32.52 (t), 34.84 (t), 36.35 (t), 51.28 (q), 52.02 (q), 73.47 (d), 101.08 (s), 126.61 (d), 126.95 (d), 130.21 (d), 130.37 (d), 131.73 (d), 132.18 (d), and 169.98 (s); LR EIMS 348 (M - O_2, 9), 289 (2), 261 (1.5), 183 (4), 155 (3.5), 153 (3), 135 (4.5), 123 (10), 121 (8), 113 (35), 111 (10), 109 (15), 107 (11), 97 (13), 95 (35), 93 (16), 81 (100), 79 (34), 68 (38), 67 (47), 59 (27), 55 (54), 53 (23), and 41 (58 rel.%).

ACKNOWLEDGMENTS

We thank Dr. F. Koehn for recording some of the NMR spectra and Dr. K. Bock for the NOE measurement on a Bruker AM 500 spectrometer donated by the Carlsberg Foundation and The Danish Natural Science Research Council. We are also grateful to Drs. M. Lui and W. Thompson for the antitumor testings and to Dr. O. S. Tendal for identification of the sponge. This work was initiated when one of us (CC) visited University of the Ryukyus as a recipient of a fellowship from Japan Society for Promotion of Science.

REFERENCES AND NOTES

1. A. A. Gunatilaka, Y. Gopichand, F. J. Schmitz and C. Djerassi, *J. Org. Chem, 46,* 3860 (1981).
2. W. Fenical, *J. Am. Chem. Soc., 96,* 5580 (1974).
3. Y. Kashman and M. Rotem, *Tetrahedron Lett.*, 1707 (1979).
4. M. Albericci, M. Collart-Lempereur, J. C. Braekman, D. Daloze, B. Tursch, J. P. Declercq, G. Germain and M. van Meerssche, *Tetrahedron Lett.*, 2687 (1979). A. Albericci, J. C. Braekman, D. Daloze and B. Tursch, *Tetrahedron, 38,* 1881 (1982).
5. L. V. Manes, G. J. Bakus and P. Crews, *Tetrahedron Lett., 25,* 931 (1984).
6. R. J. Capon and J. K. MacLeod, *Tetrahedron, 41,* 3391 (1985).
7. R. J. Wells, *Tetrahedron Lett.,* 2637 (1976).
8. M. D. Higgs and D. J. Faulkner, *J. Org. Chem., 43,* 3454 (1978).
9. D. B. Stierle and D. J. Faulkner, *J. Org. Chem., 44,* 964 (1979).
10. D. B. Stierle and D. J. Faulkner, *J. Org. Chem., 45,* 3396 (1980).
11. D. W. Phillipson and K. L. Rinehart, Jr., *J. Am. Chem. Soc., 105,* 7735 (1983).
12. S. Sokoloff, S. Halevy, V. Usieli, A. Colorni and S. Sarel, *Experientia, 38,* 337 (1982).
13. For examples, see J. J. Barchi, Jr., R. E. Moore and G. M. L. Patterson, *J. Am. Chem. Soc., 106,* 8193 (1984). C. P. Falshaw, T. J. King, S. Imre, S. Islimyeli, R. H. Thomson, *Tetrahedron Lett., 21,* 4951 (1980).
14. E. Quiñoà, E. Kho, L. V. Manes, P. Crews and G. J. Bakus, *J. Org. Chem.*, in press. We thank Professor Crews for a preprint.
15. M. N. de Laubenfels, *Oregon State College Monogr. Stud. Zool., 7,* 2471 (1954).
16. C. Lévi, *Ann. Inst. Oceanogr. Monaco (n.s.), 34,* 14 (1958).
17. M. D. Corbett and R. Chipko, *Experientia, 35,* 718 (1979).
18. D. G. Baden and M. D. Corbett, *Comp. Biochem. Physiol., 64B,* 279 (1979).
19. Ito Multi-layer Coil Separator-Extractor Model #1, P. C. Inc.