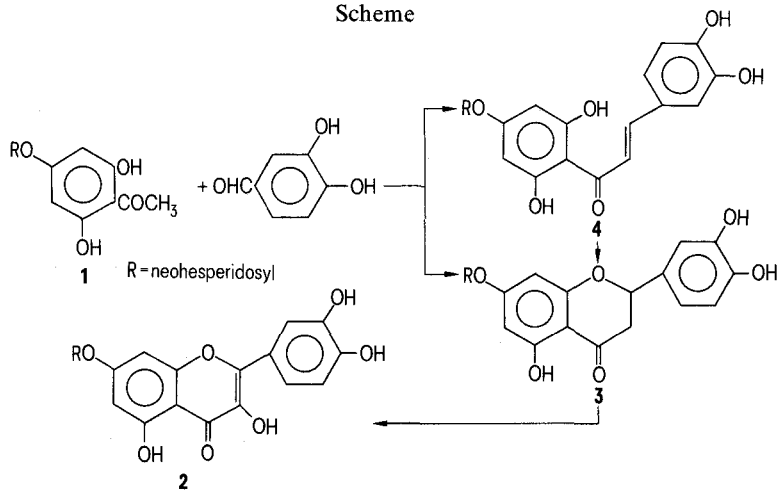
Scheme



hydrogen transfer. Thereafter a CO is lost from the m/e 357 fragment with a simultaneous formation of a phenylbenzofuran derivative at m/e 329 (20%). The neohesperidoside ion appears at m/e 393 (4%). Fragments produced by a retro-Diels-Alder fragmentation are here also of low intensity[9].

Acetylation of **2** with dry pyridine and acetic anhydride at room temperature overnight gave colorless needles (88%), recrystallized from ethanol/water: m.p. 132–134 °C; IR (KBr) ν 1755 (acetyl C=O), 1650 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.21 (d, 3H, J=6.0 Hz, rhamnose-CH_3), 1.96, 1.98, 2.02, 2.04, 2.12, 2.14 (each singlet for 3H corresponding to acetyl groups at glucose and rhamnose), 2.32 (s, 9H, 3′-, 4′- and 5-OAc), 2.42 (s, 3H, 3-OAc), 3.80–4.28 (m, 5H, glucose-H-2,5,6,6 and rhamnose-H-5), 4.90–5.46 (m, 7H, glucose-H-1,3,4 and rhamnose-H-1,2,3,4), 6.71 (d, 1H, J=2.2 Hz, 6-H), 7.01 (d, 1H, J=2.2 Hz, 8-H), 7.32 (d, 1H, J=8.5 Hz, 5′-H), 7.60–7.72 (m, 2H, 2′- and 6′-H). Anal. calculated for $C_{47}H_{50}O_{26}$: C, 54.76; H, 4.89; found: C, 54.78; H, 4.98.

1 J. Gripenberg, in: The Chemistry of Flavonoid Compounds, p.409. Ed. T.A. Geissman. The MacMillan Co., New York 1962.
2 M. Nogradi, L. Farkas, H. Wagner and L. Hoerhammer, Chem. Ber. *100*, 2783 (1967).
3 R.M. Horowitz and B. Gentili, Tetrahedron *19*, 773 (1963).
4 J. Chopin and G. Dellamonica, C. r. Acad. Sci. C *262*, 1712 (1966).
5 H. Inouye, Y. Aoki, H. Wagner, L. Hoerhammer, G. Aurnhammer and W. Budweg, Chem. Ber. *102*, 3009 (1969).
6 H. Pacheco and A. Grouiller, Bull. Soc. chim. Fr. *10*, 3212 (1966).
7 F.M. Dean and V. Podimuang, J. chem. Soc. 1965, 3978.
8 H. Wagner and O. Seligmann, Tetrahedron *21*, 3029 (1973).
9 R.D. Schmid, Tetrahedron *28*, 3259 (1972).
10 S. Hakamori, J. Biochem. *55*, 205 (1964).

Scalarolbutenolide, a new sesterterpenoid from the marine sponge *Spongia nitens*[1]

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Summary. A new sesterterpenoid, scalarolbutenolide *(5)*, has been isolated from the marine sponge *Spongia nitens;* its structure, including the absolute stereochemistry, has been established by chemical and spectroscopic studies.

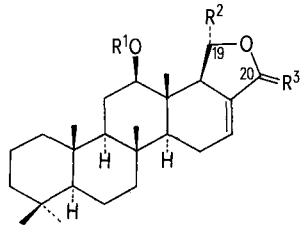
Previous reports[3–5] from this laboratory described the structures of a variety of terpenes isolated from the marine sponge *Spongia nitens.* Recently we reported[4,5] the isolation and the structures of four tetracarbocyclic sesterterpenes **(1, 2, 3, 4)** all belonging to the series of the scalarinlike[6] compounds. Continuing in this field we now report the full structure of a novel sesterterpenoid **(5),** named scalarolbutenolide.

Extraction of fresh tissues of *S. nitens,* with acetone followed by silica gel fractionation[3] of the ether soluble portion, yielded, in addition to the previously-reported terpenes, crystalline scalarolbutenolide (**5,** 0.005% of dry material, m.p. 220–222 °C, $[\alpha]_D$ ($CHCl_3$)+1.9°, TLC R_f 0.6 light petroleum - diethyl ether (2:8). The structure of scalarolbutenolide **(5)** is based on the following evidence. $C_{27}H_{40}O_5$ (high resolution mass spectrometry); UV (MeOH) 217 (ε, 7.730) nm; IR ($CHCl_3$) 3575, 1735 (acetate), 1750 and 1650 (α,β-butenolide) cm^{-1}; MS 444 (M^+, 2%), 426 (5), 384 (64), 366 (10), 275 (35), 257 (14), 205 (34), 191 (100), 137 (84), 123 (93); PMR ($CDCl_3$) 6.0 (H-20. bs, w/2 3Hz), 5.8 (H-16, m, w/2 5Hz), 4.76 (H-18, bs, w/2 3Hz), 3.80 (H-12, dd, J=4, 1OHz), 2.1 (CH_3CO, s), 0.90 (9H, s), 0.84 (3H, s), 0.68 (3H, s)δ; the CMR data are reported in the table. The mass data, compared with those of the previously described terpenes[4,5], suggest a tetracarbocyclic scalarin-like skeleton for **5,** which also has to contain an acetoxy group and an hydroxy group because of the presence in the mass spectrum of peaks due to consecu-

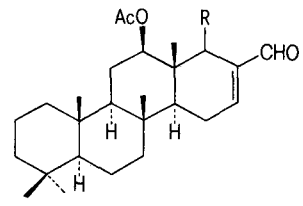
tive losses of acetic acid and water from the very weak molecular ion. The fragment ions at m/z 275 (13–18 and 14–15 cleavages $-1H$), 257 (275 $-H_2O$), and 205 (11–12 and 8–14 cleavages $-1H$) localize the hydroxy group on the ring C and, more exactly, on C(12). This assignment is confirmed by the presence in the PMR spectrum of the signal at 3.80 δ showing the typical shape[7] of the axially oriented H at C(12).

The suggested tetracarbocyclic skeleton is confirmed by the comparison (table) of the CMR spectra of **5** and its acetyl derivative **6** with that previously reported[4] for **7** and with that of the deacetyl derivative **(8)** of **7**. The signals corresponding, in the CMR spectrum of **5**, to the carbon atoms of the rings A, B and C and of the 5 methyl groups on these rings are readily recognized. The acetylation of **5** produces small upfield changes in the chemical shifts of both the carbons α and β to the hydroxy group. These unusual shifts could be justified by the presence of a hydrogen linkage between the oxygen on C(12) and the substituent on C(18). The signal due to the C(14) methyne group of **5**, compared with that of **8**, shows an upfield shift due to a γ-gauche interaction with an axially α-oriented substituent of the ring D.

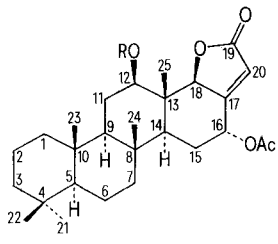
As the substituent at C(18), according to the strongly upshifted resonance of the C(25) angular methyl group, must be equatorially oriented, the group interacting with the 14-methyne must be localized on C(16). The remaining CMR signals of **5** are assigned, as reported in the table, on the basis of their chemical shifts, multiplicity and with the aid of some selective ^{13}C-[^1H] decoupling experiments for C(12), C(16) and C(18). The presence in **5** of the β,γ-disubstituted-α,β-butenolide ring, suggested by the IR and UV data along with the PMR evidence showing mutual long-range couplings among H-atoms at C(16), C(18) and C(19), was definitively confirmed by alkaline hydrolysis of **5** which gave the acid **9**, characterized as methyl ester **(10)**.



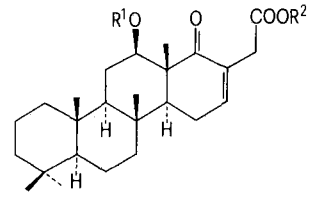
1, $R^1 = COCH_3$, $R^2 = OH$, $R^3 = O$
2, $R^1 = COCH_3$, $R^2 = OH$, $R^3 = H_2$
7, $R^1 = COCH_3$, $R^2 = H$, $R^3 = O$
8, $R^1 = H$, $R^2 = H$, $R^3 = O$



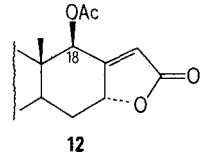
3, R = β-CHO
4, R = α-CHO



5, R = H
6, R = $COCH_3$



9, $R^1 = H$, $R^2 = H$
10, $R^1 = H$, $R^2 = CH_3$
11, $R^1 = COCH_3$, $R^2 = CH_3$



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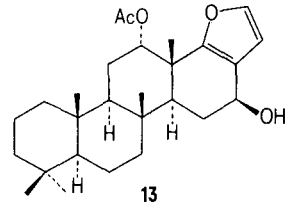

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CMR chemical shifts (in ppm from internal TMS) of compounds **5**, **6**, **7**, **8**, **10** and **11**

C	5	6	7[4]	8	10	11
C(1)	40.0	39.8	39.8	40.1	39.8	39.8
C(2)	18.2[a]	18.2[a]	18.0[a]	18.1[a]	18.0[a]	18.0[a]
C(3)	42.0	41.9	42.0[b]	41.6[b]	42.0[b]	42.0[b]
C(4)	33.3	33.3	33.3	33.3	33.2	33.2
C(5)	56.6	56.4	56.5	56.6	56.5	56.4
C(6)	18.6[a]	18.5[a]	18.5[a]	18.6[a]	18.5[a]	18.4[a]
C(7)	42.0	41.9	41.5[b]	42.1[b]	40.8[b]	40.8[b]
C(8)	37.3[b]	37.3[b]	37.4	37.5	38.4[c]	38.4[c]
C(9)	58.1	58.3	58.4	58.9	57.9	57.1
C(10)	37.6[b]	37.6[b]	37.4	37.5	37.4[c]	37.4[c]
C(11)	25.3	24.0	23.4[c]	27.8	24.8	23.8
C(12)	80.7	79.5	82.7	81.2	74.7	74.6
C(13)	47.5	46.8	38.8	40.0	49.9	48.4
C(14)	47.8	47.8	53.6	53.5	52.3	53.0
C(15)	26.9	26.4	23.7[c]	23.8	23.4	23.4
C(16)	65.9	65.9	135.6	135.1	148.3	144.8
C(17)	162.8	162.1	126.5	127.2	130.6	130.9
C(18)	89.5	87.6	49.7	50.7	–	–
C(19)	169.2	169.2	67.7	69.1	171.5	172.2
C(20)	116.8	117.3	169.4	169.0	35.3	35.2
C(21)	33.3	33.3	33.3	33.3	33.2	33.3
C(22)	21.3[c]	21.4[c]	21.4[d]	21.4	21.3	21.3
C(23)	16.4	16.4	16.6	16.7	16.3	16.3
C(24)	17.1	17.3	16.6	16.7	17.6	17.2
C(25)	6.5	6.9	9.0	7.9	12.0	12.7
OCH_3	–	–	–	–	51.9	53.0
CH_3CO	170.4	170.1[d]	170.1	–	–	170.6
CH_3CO	21.1[c]	21.4[c]	21.3[d]	–	–	21.3
CH_3CO	–	171.9[d]	–	–	–	–
CH_3CO	–	21.2[c]	–	–	–	–

Spectra were determined in $CDCl_3$ on a Varian XL-100 F.T. spectrometer operating at 25.20 MHz. [a–d] Signals may be reserved.

[M.p. 198-199 °C; $[\alpha]_D$ ($CHCl_3$) = 11.6°; UV (MeOH) 234 (6800) nm; IR ($CHCl_3$) 3500, 1725, 1650 cm^{-1}; MS 416 (M^+); PMR (C_6D_6) 6.24 (H-16, m; w/2 10Hz), 3.92 (H-12, dd, J = 4, 10Hz), 3.36 ($-OCH_3$, s), 3.06 [CH=C−CH_2−CO, ABq, J = 15Hz, 2H long-range coupled with H at C(16)]; the CMR data are reported together with those of the acetyl derivative **11** in the table].

The above arguments lead to the allocation of the structure **5** to the new sesterterpenoid. The localization of the acetoxy group on C(16) is suggested by the easy elimination, observed in the mass spectrum of acetic acid, which could not occur in the alternative structure **(12)** showing the acetoxy group on C(18). The relative stereochemistry of scalarolbutenolide has been established, as shown in formula **5**, according to previous findings for scalarin-like compounds[4,5,8,9] along with the configurational arguments reported throughout the text. The Horeau method[10] applied to **5** allows to determine the chirality of C(12) as R, and this determines its absolute stereochemistry.

Scalarolbutenolide shows the same tetracarbocyclic skeleton of the scalarin-like compounds with different arrangements of the carbons C(19) and C(20). The same skeleton as **5** has been previously encountered only in furoscalarol **(13)**[11,9].

1 This work is a part of the 'Progetto Finalizzato per l'Oceanografia e i Fondi Marini', C.N.R., Roma.
2 We are indebted to A. Crispino, C. Di Pinto, G. Scognamiglio and R. Turco for their technical assistance.
3 E. Fattorusso, L. Minale, G. Sodano and E. Trivellone, Tetrahedron *27*, 3909 (1971).
4 G. Cimino, S. De Stefano, L. Minale, and E. Trivellone, J. chem. Soc. Perkin I, *1977*, 1587.
5 G. Cimino, S. De Stefano and A. Di Luccia, Experientia *35*, 1277 (1977).
6 E. Fattorusso, S. Magno, C. Santacroce and D. Sica, Tetrahedron *28*, 5993 (1972).
7 E.R.H. Jones, 8th Symp. Chem. Natural Products, New Delhi, February 1972, p. 43. Ed. T.R. Govindachari. Butterworths, London 1972.
8 Y. Kashman and A. Rudi, Tetrahedron *33*, 2997 (1977).
9 G. Cimino, F. Cafieri, L. De Napoli and E. Fattorusso, Tetrahedron Lett. *1978*, 2041.
10 A. Horeau, Tetrahedron Lett. *1961*, 506; *1962*, 965.
11 F. Cafieri, L. De Napoli, E. Fattorusso, C. Santacroce and D. Sica, Gazz. chim. ital. *1977*, 1587.

The synthesis of enantiomers of 4-ketocyclophosphamide

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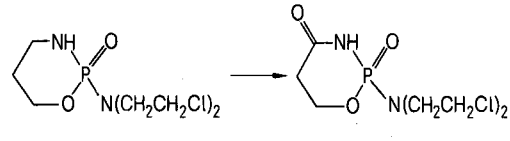
Summary. Oxidation with Fenton's reagent and by direct ozonation of the enantiomers of the antitumour agent cyclophosphamide afforded optically pure enantiomers of 4-ketocyclophosphamide. Oxidation with potassium permanganate, however, gave the corresponding racemate.

As part of an investigation into the stereoselective metabolism of cyclophosphamide (**1**) and its congeners in experimental animals[1] and in man[2], a study of the stereospecific synthesis of the parent drugs[3] and their metabolites was undertaken. In this communication we report on the synthesis of the enantiomers of 4-ketocyclophosphamide (**2**). Cyclophosphamide (**1**) is readily oxidized by $KMnO_4$ to **2**[4], but when cyclophosphamide enantiomers were similarly treated, only racemic **2** was obtained.

However, oxidation of optically pure $R(+)$-**1** with Fenton's reagent ($FeSO_4/H_2O_2$)[5] afforded $R(-)$-**2** as the thick oil in 4.5% yield $|\alpha|_D^{25} = -30°$; unreacted $R(+)$-**1** with unchanged optical rotation was recovered. Although the chemical and optical purity of the above product **2** has been proved by ^{31}P NMR spectroscopy in the presence of Eu(tfc)$_3$ reagent, by mass spectrometry and by several chromatographic techniques, attempts to crystallize it have failed. For purposes of comparison 4-ketocyclophosphamide was prepared by ozonation[6] of $S(-)$-**1**. The crystals of $S(+)$-**2** m.p. 107 °C (from ethyl-ether), were obtained $|\alpha|_D^{25} = +53.8°$ (c 3, MeOH) in 16% yield. The enantiomeric purity of at least 97% was demonstrated using the ^{31}P NMR/Eu(tfc)$_3$ technique. Because the absolute configuration of the starting cyclophosphamide was known[7], and no bond to the chiral phosphorus atom was cleaved, the absolute configuration of (+)-**2** ($|\alpha|_D^{25} = +53.8°$) is *S*. It should also be emphasized that oxidation by Fenton's reagent is a process which mimics in some respects the biological oxidations mediated by the liver mixed function oxidases[8]. It would therefore be predicated that racemization of enantiomeric cyclophosphamides should not occur during oxidative metabolism.

$S(+)$-4-Ketocyclophosphamide $|S(+)$-**2**$|$. Gaseous ozone (Ozon Generator, Fischer 501) was bubbled at 0 °C at a rate of 1 g/h through a solution of $S(-)$-cyclophosphamide (**1**, $|\alpha|_D^{25} = -2.3°$, 3.83 mmole) in acetone (9 ml) and water (18 ml). After 8 h the ^{31}P NMR spectrum of reaction mixture revealed the presence of **2** (20%, $\delta = 6.5$ ppm), 4-hydroperoxycyclophosphamide (20%, $\delta = 10.2$ ppm) and unreacted **1** (60%, $\delta = 14.3$ ppm). Further ozone did not change the composition of the mixture. After evaporation of the acetone, the aqueous solution was extracted with chloroform (3 × 25 ml). The product was isolated by column chromatography on silica gel with chloroform-acetone (1:1) as an eluent. $S(+)$-**2** was recrystallized from ethyl ether to obtain 168 mg (16% yield) as white crystals |m.p. 107 °C $|\alpha|_D^{25} = +53.8°$ (c 3, MeOH), $R_f = 0.59$ (chloroform-acetone 1:3), $\delta_{31P} = 5.0$ ppm ($CHCl_3$, downfield from



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