on charcoal yielded a new product identical in gas chromatographic retention times to hexadecan-1-ol acetate on columns 4 and 5. These data suggested that the pheromone is the acetate of a 16-carbon, straight-chain alcohol.

The ratio of the gas chromatographic retention time of the sex pheromone to that of hexadecan-1-ol acetate was 0.87 on column 3 (SE-30), 1.21 on column 2 (Carbowax 20 *M*), and 1.43 on column 4 (DEGS). By comparison, the ratios of z-7-hexadecen-1-ol acetate to hexadecan-1-ol acetate were 0.91, 1.08, and 1.18 on the same 3 columns. The longer retention times on the polar columns of the pheromone compared with z-7-hexadecen-1-ol acetate suggested that the pheromone has more than one double bond.

A mass spectrum of the purified sex pheromone obtained from a mass spectrometer equipped for chemical ionization showed the following diagnostic peaks: A cluster of ions at *m/e* 281, 280, and 279 resulting from $(P + 1)^+$, P^+, and $(P - 1)^+$, respectively; *m/e* 221, $[(P + 1) - CH_3COOH]^+$; *m/e* 61, $(CH_3COOH_2)^+$; and *m/e* 123, base peak[5]. These data confirmed that the sex pheromone is a 16 carbon-alcohol acetate with 2 double bonds.

Microozonolysis[6] of 1-µg quantities of the sex pheromone in hexane (99% mole pure) yielded a compound with GC retention times on columns 2, 3 and 4 identical to those of 7-oxoheptyl acetate formed by the ozonolysis of Z-7-hexadecen-1-ol acetate. A second fragment formed during the ozonolysis of the sex pheromone had retention times identical to those of valeraldehyde on columns 2, 4, and 6. Therefore, the sex pheromone was identified as one of the 4 possible isomers of 7, 11-hexadecadien-1-ol acetate.

An active isomer, (Z, E)-7, 11-hexadecadien-1-ol acetate, was synthesized from 3-octyn-1-ol and 6-chloro-1-hexanol as starting materials with a 7.1% overall yield by the scheme outlined in Figure 1. The other 3 isomers were also synthesized by analogous procedures but elicited almost no biological activity from the males. The detailed synthesis will be reported elsewhere. The isomeric identity of the synthetic pheromone was confirmed by spectrometric means[7]. Diagnostic peaks of the mass spectrum and GC retention times on columns 2, 4, and 5 were identical for Z-7-, E-11-hexadecadien-1-ol acetate and for the isolated natural sex pheromone.

No significant differences between male responses to the natural sex pheromone and to (Z, E)-7, 11-hexadecadien-1-ol acetate could be detected by laboratory bioassay (Figure 2, paired $t = 0.03$). A preliminary field test was run as follows: Two sticky traps, one baited with 25 ng of natural and one baited with 25 ng of synthetic sex pheromone each impregnated on filterpaper strips, were suspended against the walls of a $6 \times 6 \times 2.4$-m room at a height of 1.5 m. The locations of the two baited traps were alternated nightly for 8 nights. Each night 100 males were released into the center of the room midway through the 10-h scotophase. Male catches were recorded 5 h later. Used traps, baits, and untrapped insects were discarded after each test. Traps baited with natural and synthetic pheromone caught 21 ± 8 ($\bar{X} \pm SD$) and 20 ± 12 males, respectively, each night. Untreated control traps tested simultaneously with the treated traps caught 3.5 ± 2 males per night. The difference between the treatment means was not significant ($t = 0.25$). We therefore conclude that (Z, E)-7, 11-hexadecadien-1-ol acetate is the sex pheromone produced by the female Angoumois grain moth.

Zusammenfassung. Der Sexuallockstoff der Angoumois Getreidemotte, *Sitotroga cerealella* (Olivier), wurde als (Z, E)-7, 11-Hexadecadien-1-ol Acetat identifiziert. Die synthetisierte Verbindung ist chromatographisch, spektroskopisch, und verhaltensmässig identisch mit dem von den Weibchen produzierten Sexuallockstoff.

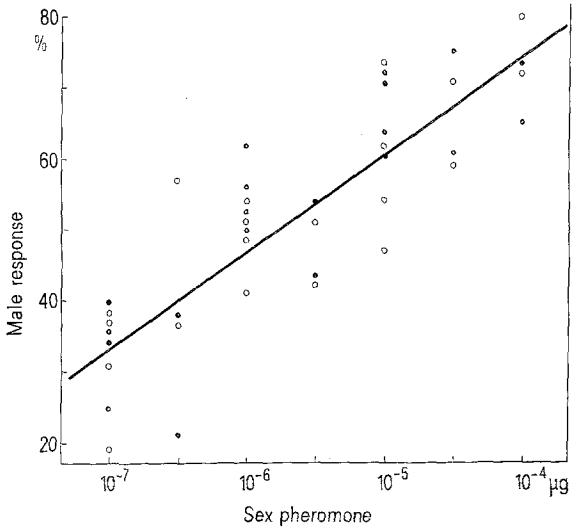

Fig. 2. Responses of male Angoumois grain moths to the synthetic pheromone (solid circles) and the natural pheromone (open circles). The 2 treatments were not significantly different ($t = 0.03$ at 19 df).

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[5] We thank J. H. TUMLINSON, Insect Attractants Laboratory, Gainesville, Fla., and J. NELSON and F. MATSUMURA, Entomology Dept., University of Wisconsin, Madison, Wisconsin, for the mass spectra of the isolated female sex pheromone.

[6] M. BEROZA and B. A. BIERL, Analyt. Chem. *39*, 1131 (1967).

[7] We thank J. H. TUMLINSON and R. R. HEATH for confirming the location and configuration of the double bonds in the synthetic sex pheromone. The double bonds were individually epoxidized and examined by IR, NMR, and chemical ionization mass spectrometry. This technique will be published shortly.

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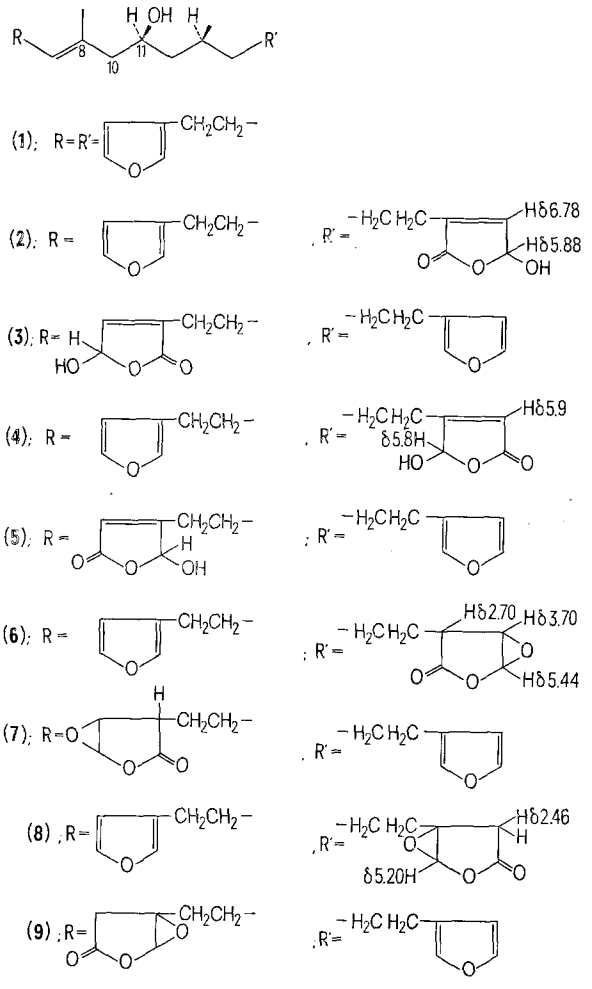
Oxidized Furanoterpenes from the Sponge *Spongia officinalis*

Previous work[1,2] on the sponge *Spongia officinalis* has resulted in the isolation of 6 closely related linear C_{21}-difuranoterpenes, all possessing the same carbon skeleton. An accurate analysis of the more polar fractions from the methanolic extracts of *S. officinalis* has now led to the isolation, in small amount, of 4 C_{21}-monofuranoterpenes, closely related to furospongin-1 (**1**)[1], the major C_{21}-terpene component of the same sponge, with 1 furan ring



modified as a γ-hydroxy-α, β-butenolide (**2**–**5**). In addition we have also obtained a mixture of 4 more less-polar β, γ-epoxybutenolides (**6**–**9**) which very readily undergoes decomposition to the previous γ-hydroxy-α, β-butenolides (**2**–**5**).

Extraction of *Spongia officinalis* (350 g, dry wt. after extraction) and the subsequent SiO_2 column chromatography were described previously[1]; after removing the C_{21}-difuranoterpenes by washing the column with benzene, elution with benzene and increasing amounts of ether yielded the β, γ-epoxybutenolide (ca. 50 mg) and the γ-hydroxy-α, β-butenolide (ca. 200 mg) fraction, in that order.

γ-Hydroxy-α,β-butenolide fraction (**2**–**5**). This was resolved into 2 Ehrlich-positive fractions, in approximately equal proportions, by TLC on silica gel in benzene: ether (4:1), Rf 0.3 and 0.2, respectively. Attempts to obtain single components were fruitless. Both fractions gave substantially identical MS with a molecular ion at *m/e* 362 and prominent peaks at *m/e* 213, 181 and 150, IR with absorptions for OH (3450–3200 cm^{-1}), CO (1755 cm^{-1}) and double bonds (1640 cm^{-1}), and UV (λ_{max} 223 nm, log ε = 3.96). The NMR-spectra of both fractions showed signals corresponding to those assigned in the spectrum of furospongin-1 (**1**) to a β-substituted furan ring (1H broad singlets at δ 7.25, 7.13 and 6.18), a CHOH group (1H, broad multiplet at δ 3.70, H–11), a vinyl methyl (δ 1.61, d, J = 2Hz), and a *sec*-methyl (0.89, J = 6Hz).

In the low-field region of the spectrum, the less-polar fraction, Rf ca. 0.3, showed 2 broad doublets (1H each, J = 2 Hz) at δ 6.78 and 5.88, which can be assigned to an α-substituted-γ-hydroxy-α,β-butenolide system[3,4] in agreement with the IR- and UV-data, and with the formation of a diacetate[5] on treatment with acetic anhydride in cold pyridine. All these data can reasonably be interpreted in terms of the structures (**2**) and (**3**) for the less-polar fraction, in which one of the β-substituted furan rings of furospongin-1 (**1**) is modified as a α-substituted-γ-hydroxy-α,β-butenolide. The fragmentation pattern in the MS confirmed that this fraction is actually a mixture: peaks at *m/e* 150[6] and 213 are associated with (**2**), originating by cleavage of the 10, 11-bond, while the peak at *m/e* 181 is associated with (**3**) arising by cleavage of the same 10,11-bond.

The mixture of isomers (**2**) and (**3**) was directly correlated with furospongin-1 (**1**). $NaBH_4$ reduction of the mixture (**2**) and (**3**) gave a mixture of the corresponding α, β-unsaturated γ-lactones, which was characterized as mixed acetates[7]. The γ-lactone function was reduced with di-isobutyl aluminium hydride in tetrahydrofuran[8], to afford a difurano derivative indistinguishable from furospongin-1 acetate by TLC, GLC (1% SE-30°) and NMR.

The slightly more polar fraction, Rf ca. 0.2, contained a mixture of (**4**) and (**5**), in which one of the furan rings of furospongin-1 (**1**) is oxidized to a β-substituted-γ-hydroxy-α, β-butenolide. The 100 MHz NMR-spectrum is remarkably similar to that of furospongin-1 (**1**), except that the resonances arising from one β-furan ring in the latter are replaced by those of a vinyl and a *CH*OH proton at δ 5.9 and 5.8, respectively (each a broad singlet; see formulae **4** and **5**)[3]. Chemical confirmation was similary achieved by direct correlation[9] with furospongin-1 (**1**).

β,γ-Epoxybutenolide fraction (**6**–**9**). This fraction shows IR-absorption for a saturated γ-lactone ring (1790 cm^{-1}), while the UV-spectrum exhibits a maximum at 216 nm, log ε = 3.45 (furan chromophore). Its NMR-spectrum has the same features as that of furospongin-1 (**1**) except that resonances arising from one β-substituted furan ring in (**1**) are replaced by signals at δ 5.44 (d, J = 2 Hz, 0.5H), 5.20 (m, 0.5H), 3.70 (t, J = 2Hz, 0.5H), 2.70 (m, 0.5H) and 2.46 (m, 1H), thus suggesting

[1] G. Cimino, S. de Stefano and L. Minale, Tetrahedron *27*, 4673 (1971).

[2] G. Cimino, S. de Stephano and L. Minale, Tetrahedron *28*, 267 (1972).

[3] J. M. Ferland, Y. Lefebvre, R. Deghenghi and K. Wiesner, Tetrahedron Lett. *1966*, 3617.

[4] B. A. Burke, W. R. Chan, K. E. Magnus and D. R. Taylor, Tetrahedron *25*, 5007 (1969).

[5] The mixture of the acetates of (*2*) and (*3*) has the following spectra features: MS, *m/e* 446 (M^+), 386, 326; IR, ν_{max} 1770 and 1728 cm^{-1}; NMR, δ 6.78 (2H, broad singlet, protons of the butenolide moiety)[4], 2.1 and 1.92 (each 3H singlets, CH_3CO–).

[6] It should be noted that the peak at *m/e* 150 originates by cleavage of the 10, 11-bond and H transfer; the same strong peak is equally apparent in the mass spectrum of furospongin-1 (*1*)[1].

[7] The mixture of α, β-unsaturated γ-lactone acetates derived from (*2*) and (*3*) has the following properties: MS, M^+/*e* 388; NMR, δ 7.10 (1H, t, J = 2 Hz, –CH=C–C=O) and 4.68 (2H, narrow multiplet, $-CH_2$–O–C=O)[4] coupled to each other, and confirmed by decoupling experiments; IR ν_{max} 1750 (α, β-unsaturated γ-lactone), 1730 (acetate) and 1690 (C=C).

[8] H. Minato and T. Nagasaki, J. chem. Soc. (C) *1966*, 377.

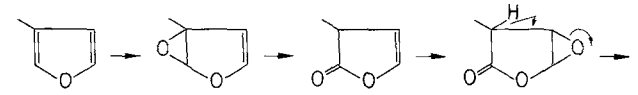
[9] The mixture of α, β-unsaturated γ-lactones, obtained by $NaBH_4$ reduction of the mixture of lactols (*4*) and (*5*), was also characterized as the acetates: MS, M^+/*e* 388; NMR, δ 5.74 (1H, narrow multiplet, C=C*H*–C=O)[10] and 4.61 (2H, narrow multiplet, $-CH_2$–C=O); IR, ν_{max} 1750 (α, β-unsaturated γ-lactone), 1730 (acetate) and 1685 (C=C).

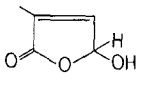
that this fraction is a mixture of a least two components. This was confirmed by the MS, which showed the same molecular ion (*m/e* 362; 7%) as that of the γ-hydroxy--α, β-butenolides and an identical fragmentation pattern with strong peaks at *m/e* 213 (80%), 181 (75%) and 150 (100%)[11]. Attempts at separation by SiO_2 chromatography resulted in the isolation of the mixture of (**2–5**), as indicated by IR and NMR. Acetylation of (**6–9**) gave the mixture of γ-acetoxy-α, β-butenolide acetates. All these data are constitent with structures (**6–9**), and the NMR-signals, listed above, can be reasonable assigned to the β, γ-epoxybutenolide moieties as indicated in formulae (**6**) and (**9**). Protons at δ 5.44 and 3.70 are coupled to each other, as confirmed by decoupling experiments.

Terpenoids with different states of oxidation of the furan ring are known[4,12,13], but their status as natural products is uncertain. It is established[14–16] that alkylated furans give autoxidation products of type (**10**), and evidence has been accumulated that such photo-oxidations proceed via ozonides, which, in a few cases, have been isolated. Moreover, the co-occurrence in a specimen of *Cedrela odorata* of gedunin and photogedunin, in which latter the furan ring is oxidized to a γ-hydroxy-α, β-butenolide, has been reported recently[4].

The mode of isolation of photogedunin, and its absence from other samples of *Cedrela* in which gedunin was present, led the authors[4] to suggest that the photo-oxidation of the furan ring might occur in vivo.

Extraction of a sample of *Spongia officinalis* in the dark gave both γ-hydroxy- and β, γ-epoxybutenolide fractions, while from a methanolic solution of furospongin-**1** (**1**) we were unable to detect any of the above oxidized derivatives after exposure to light. This indicates that at least the β, γ-epoxybutenolides (**6–9**) are genuine natural products and that possibly the oxidation of the furan rings might occur through epoxidation steps as indicated in Scheme 1, in contrast to photo-oxidation in vitro[15] (the spontaneous isomerization of arene oxides to phenols is well documented[17]).





(**10**)

Finally we want to emphasise that β, γ-epoxybutenolides are compounds of a type not previously encountered.

Riassunto. L'isolamento dalla *Spongia officinalis* di otto C_{21} monofuranoterpeni, strettamente correlati alla furospongina-1 (**1**), il componente terpenico più abbondante della stessa spugna, per quattro dei quali si dimostrano le strutture di γ-idrossi-α,β-butenolidi (**2–5**) e per gli altri quattro le strutture di β,γ-epossibutenolidi (**6–9**), sembra rilevante in connessione col problema della ossidrilazione dei substrati aromatici in vivo.

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Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Via Toiano 2, Arco Felice (Napoli, Italy), 30 July 1973.

[10] N. S. BHACCA, L. F. JOHNSON and J. N. SHOOLERY, *NMR Spectra Catalog* (Varian Associates, Palo Alto, California 1962), vol. 1, No. 51.

[11] The MS of this fraction also showed significant peaks at *m/e* 135 (40%) and 195 (20%), the former associated with (*6*) and (*8*) arising by cleavage of the 8,10-bond, and the latter is associated with (*7*) and (*9*) originating by cleavage of the same 8,10-bond.

[12] L. NOVOTNY, V. HEROUT and F. SORM, Collection Czech. chem. Comm. *29*, 2182 (1964); *29*, 2189 (1964).

[13] J. D. CONNOLLY, K. L. HAUDA and R. MCCRINDLE, Tetrahedron Lett. *1968*, 437.

[14] T. KUBOTA, in *Cyclopentanoid Terpene Derivative* (Eds. W. J. TAYLOR and A. R. BATTERSBY; Marcel Dekker, Inc., New York 1969), p. 293.

[15] K. GOLLNIK and G. O. SCHENK, in *1,4-Cycloaddition Reactions* (Ed. J. HAMER; Academic Press, New York–London 1967), p. 288.

[16] C. S. FOOTE, M. T. WUESTHOFF, S. WEXLER, G. O. SCHENCK and K. M. SCHULTE-ELTE, Tetrahedron *23*, 2583 (1967).

[17] J. W. DALY, D. M. JERINA and B. WITKOP, Experientia *28*, 1129 (1972) and references therein.

[18] Acknowledgements. We are indebted to Professor R. H. THOMSON (University of Aberdeen, Scotland) for his interest and advice on this problem. We also wish to thank Professor E. LEDERER and Dr. B. C. DAS (Gif-sur-Yvette, France) for some mass spectra. The technical assistance of Mr. C. DI PINTO (NMR) and G. SCOGNAMIGLIO is also acknowledged.

Hemin as a Catalyst for Chemiluminescence

The chemiluminescence produced by the reaction of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in a basic solution in the presence of hydrogen peroxide and catalyzed by protohemin (protoporphyrin IX iron III chloride) has been well studied. The actual mechanism of the reaction, however, is not thoroughly understood. The purpose of this investigation was to determine the effect of the chemical modification at the 2 and 4 positions of protohemin on its ability to catalyze the decomposition of luminol.

When the vinyl side chains of hemin are modified, various electronic effects have been observed through examination of the absorption spectra in solvents of varying polarity, at different pH values and as pyridine hemochromogens. Changes in both intensity and position of the absorption maxima occur and the degree of correlation between catalytic and absorptive changes is considered.

The assays for chemiluminescence were done according to the method of NEUFELD et al[1]. The salient features of the instrument used are: a holder for the test tube which is light-tight, a camera shutter that permits changing sample tubes without turning off the high voltage to the phototube, and a housing for the phototube. We used either a RCA IP28, IP21, or an EMI 9635 phototube; amplification was provided by a Sanborn preamplifier model 150–1500. The data were recorded on a Sanborn 151 recorder. The reaction is triggered by the injection of H_2O_2 into a test tube containing alkaline luminol, EDTA, and the catalyst.

[1] H. A. NEUFELD, C. J. CONKLIN and R. D. TOWNER, Analyt. Biochem. *12*, 303 (1965).