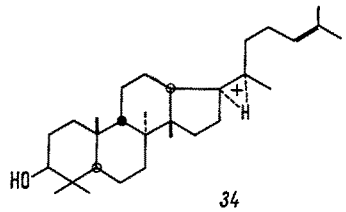

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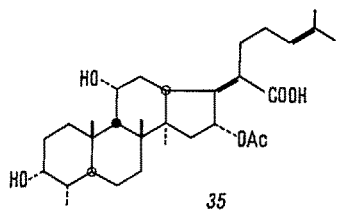

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squalene. The production of fusidic acid may accordingly be considered the result of an aberrant lanosterol biosynthesis. The ability of *Fusidium coccineum* to fold squalene in the manner required by such a scheme is borne out by the identification of ergosterol as one of the components from the mycelial lipid fraction.

Since the appearance of our last note[1], BUCOURT et al.[14] have independently confirmed the location of the hydroxyl group in ring C of fusidic acid and have advanced for the antibiotic an alternative stereochemical expression, *35*, which is incompatible with some of the results reported in the present work. We believe that the data reported by these workers, when viewed in proper perspective, constitute in fact corroborative evidence for the correctness of formula *2*.

Compound		m.p.	UV spectrum	
			λ_{max} (mμ)	ε
4	$C_{29}H_{44}O_4$	208–210°		
5	$C_{31}H_{48}O_5$	223–225°		
6	$C_{29}H_{46}O_4$	192–193°		
7	$C_{29}H_{45}O_4Br$	155–156°		
8	$C_{30}H_{46}O_5$	160–161°	292	7,600
10	$C_{29}H_{44}O_4$	211–214°	238	9,200
11	$C_{29}H_{44}O_4$	209–212°	238	6,850
12	$C_{29}H_{44}O_4$	192–194°		
13	$C_{29}H_{44}O_6$	225–226°		
14	$C_{31}H_{48}O_5$	157-158°		
16	$C_{33}H_{52}O_7$	186–187°	221	8,350
17	$C_{31}H_{48}O_6$	189–190°	220	8,150
18	$C_{29}H_{46}O_5$	183–184°	228	10,750
19	$C_{30}H_{48}O_5$	178–179°	231	10,650
21	$C_{30}H_{46}O_4$	111–112°	272	18,950
22	$C_{30}H_{48}O_4$	amorphous	234	10,650
23	$C_{30}H_{50}O_4H_2O$	94–98°	234	12,150
24	$C_{34}H_{54}O_6$	120–121°	234	12,100
25	$C_{25}H_{38}O_5$	178–179°		
26	$C_{25}H_{38}O_5$	180–181°		
27	$C_{31}H_{48}O_4$	190–191°		
28	$C_{31}H_{48}O_5$	206–207°		
29	$C_{31}H_{44}O_6$	283–286°	238	10,000
30	$C_{29}H_{42}O_5$	257–259°		
31	$C_{29}H_{40}O_5$	204–205°	238	9,600
32	$C_{29}H_{38}O_6$	225–227°	250	11,500
33	$C_{29}H_{38}O_5$	170–174°	289	12,000

Zusammenfassung. Neue chemische Umsetzungen der Fusidinsäure, sowie physikalische Messungen der dabei erhaltenen Abbauprodukte erlauben, zusammen mit früheren Ergebnissen[1,3], dem Antibiotikum die in Formel *2* wiedergegebene Stereochemie zuzuteilen. Ein möglicher Zusammenhang zwischen den Biogenesen von Fusidinsäure und Lanosterin wird kurz diskutiert.

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March 23, 1964.

[14] R. BUCOURT, M. LEGRAND, M. VIGNAU, J. TESSIER, and V. DELAROFF, C.R. Acad. Sci. *257*, 2679 (1963). We are indebted to Prof. VELLUZ for sending to us a copy of the manuscript prior to its publication.

Unsaponifiable Constituents in *Spirographis spallanzani*

What we know about the distribution of the Ubiquinones in the animal kingdom can be summarized as follows:

The higher homologues, Ubiquinones 50 and 45, have been demonstrated in mammals[1–3] and fishes[4]. The lower homologues with 6 to 8 isoprenic residues in the lateral chain, are typical of microorganisms[5,6]. Some of these, however, have Ubiquinone 50[7]. Among the insects, the blowfly larva (*Calliphora erythrocephala*) has been found to contain Ubiquinones 50, 45 and 40[8], whereas the housefly (*Musca domestica*) and the cabbage butterfly (*Pieris rapae*) have only Ubiquinone 45[6]. In the earthworm (*Lumbricus terrestris*) a quinone has been tentatively identified as Ubiquinone 50 by LESTER and CRANE[6]. Recently Ubiquinone 50 has been extracted and crystallized from sea urchin sperm[9]. The same homologue is present in the unfertilized eggs of *Paracentrotus lividus*[10].

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[3] N. F. CUNNINGHAM and R. A. MORTON, Biochem. J. *72*, 92 (1959).
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[8] D. L. LAIDMAN and R. A. MORTON, Biochem. J. *84*, 386 (1962).
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[10] G. CASERTA, Rend. Accad. Naz. Lincei, in press.

Preliminary work in this laboratory has indicated the presence of Ubiquinone compounds in several representatives of other marine invertebrate groups, like coelenterates, molluscs and tunicates.

A study of the unsaponifiable fraction obtained from the body muscle of a marine annelid, *Spirographis spallanzani*, is reported in this paper. A quinone compound having several properties in common with the Ubiquinones, has been identified together with tocopherol. However, some of the physical properties of this quinone compound are different from those of the known, naturally occurring Ubiquinones. Tocopherylquinone has also been identified in the purified fraction; it is formed from tocopherol during the purification procedure.

Material and Methods. The animals were collected from the Bay of Naples. The tube and the appendages were cut away, the body was opened longitudinally and the thin muscular layer was dissected by removing the cuticle and all the internal organs with a razor blade, under running sea water. 1 kg of muscle was obtained from about one thousand animals, the material being stored at −20°C when not used immediately.

The tissue (1 kg) was minced and homogenized in a Waring blendor, in batches of 200 g with 10% KOH in 95% ethanol containing 5% pyrogallol (1.5 l for 1 kg). The material was heated under reflux on a boiling water bath for 60 min, cooled and extracted three times each with 500 ml of heptane. The combined extracts, of a deep orange colour, were washed with distilled water until neutral, dried over anhydrous sodium sulphate, filtered and the solvent removed by distillation over vacuum. The reddish residue was redissolved with 20 ml of warm ethanol, then left in the cold overnight. The crop of crystallized sterols formed was removed by filtration, the filtrate was brought to reduced volume (4 ml) in a current of nitrogen and cooled again as before. The procedure was repeated until no further appreciable precipitation of sterols was observed.

The unsaponifiable lipids, free of sterols, were dissolved in isooctaine and chromatographed. Several of the orange-reddish components do not separate and are not removed by silicic acid or Decalso or alumina. They are partially removed by Floridin which has been used in several experiments. The material present in the extract, however, does not crystallize and can only be identified by spectrophotometry and chromatography. Better purification can be achieved by absorption on carbon (Darco). After centrifugation, the carbon was washed several times with isooctane and then eluted with chloroform. The pale yellow eluate was dried under *vacuo*, the residue dissolved in ethanol, dried again, redissolved in petrol ether and chromatographed on acid washed alumina according to CUNNINGHAM and MORTON[3]. The quinones and related compounds present in each fraction obtained from the alumina column were identified by chromatography on Whatman 3 mm paper previously impregnated with Dow silicone fluid 550 and using *n*-propanol:water 4:1 as solvent[11]. Pure samples of various Ubiquinone isoprenologues were used for comparison.

The ultraviolet absorption spectra of the extracts and of all the chromatographic fractions were taken in ethanol with a Beckman DK-2 automatic recording spectrophotometer.

The solvents used for extraction and column chromatography were pure for spectrophotometry. Crystalline Ubiquinone 50 was prepared from beef heart according to CRANE et al.[2]. Ubiquinone 30 was a gift of the Istituto Sieroterapico Italiano. Tocopherylquinone was prepared from a pure sample of tocopherol according to KARRER and GEIGER[12]. The product was subjected to unidimensional chromatography as already described. The Ubiquinone compounds were further identified by the α,α'-dipyridil reagent and the CRAVEN test[13]. Tocopherol was also identified colorimetrically according to FURTER and MEYER[14].

Results. (a) Extract purified by treatment with Floridin: When the unsaponifiable material from Spirographis muscle, free of sterols, is dissolved in 6 ml benzol and treated for 10 min with Floridin (2 g), the supernatant still contains orange coloured pigments. The extract is dried, then redissolved in ethanol. By further absorption on alumina and elution with mixtures of ether and light petrol, however, a fraction is obtained (2% petrol) in which chromatography, spectrophotometry and colorimetric methods all showed tocopherol to be present. No quinone compounds were detectable in any fraction of the extract, probably being masked by the coloured pigments.

(b) Extract purified by absorption with carbon: When the unsaponifiable material in 10 ml of light petrol is washed on the column by more petrol ether (700 ml), a substance which shows in ethanol a band with a maximum at 258 mμ is observed. No change of this band occurs after addition of KBH_4. By elution with mixtures of ethyl-ether-petrol ether at increasing concentrations of ethyl ether from 4 to 100%, several coloured bands separate on the column. Samples of 10 ml each are collected at a rate of 2.5 ml per min; they are dried under nitrogen and the residues are redissolved in ethanol. Only for two fractions, which are eluted by 50% ether in light petrol and with 100% ether, the ultraviolet spectrum changes after reduction. These fractions were called S_1 and S_2 respectively.

Fraction S_1 exhibits an UV-absorption band with peaks at 261 and 269 mμ which are typical of tocopherylquinone (Figure 1). This band disappears after reduction with borohydride and a new one, with maximum at 286 mμ, is

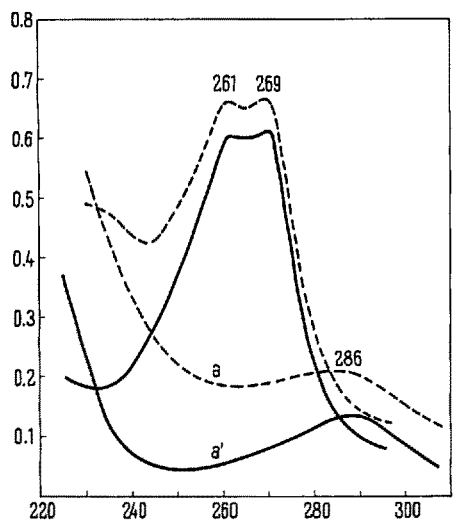

Fig. 1. Ultraviolet spectrum S_1 (– – –) as compared with synthetic tocopherylquinone (——). a and a', after reduction with KBH_4.

[11] R. L. LESTER and T. RAMASARMA, J. biol. Chem. *234*, 673 (1959).
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[13] R. CRAVEN, J. chem. Soc. *1931*, 1605.
[14] M. FURTER and R. E. MEYER, Helv. chim. Acta *22*, 240 (1939).

observed, due to the formation of tocopherylhydroquinone. Samples of this fraction were chromatographed on paper. After treatment with borohydride and development with 2,3,5-triphenyltetrazole in 0.2 *M* phosphate buffer pH 7, one single spot was observed at the identical position of synthetic tocopherylquinone.

The fraction S_2, redissolved in a minimum amount (0.5 ml) of ethanol, gives a crop of yellow crystals at −20°C. These were recrystallized from a smaller quantity (0.05 ml) of ethanol and were found to have a melting point at about 4–5°C and a spectrum in ethanol with bands at 279 mμ and 405 mμ. After reduction with borohydride the band in the visible region disappears and the peak in the UV is shifted to 290 mμ (Figure 2).

Paper chromatography of this fraction was run together with samples of pure Ubiquinone homologues. A spot was observed which has a Rf higher than Ubiquinone 30. CRAVEN's reaction for Ubiquinone was positive and gave the typical blue colour.

Discussion and Conclusions. The unsaponifiable fraction from the muscle tissue of *Spirographis spallanzani* contains a number of coloured substances which are not removed by treatment with Floridin, Decalso, Silicic acid or Alumina. A good purification of the extract can be obtained by absorption on carbon. This treatment, however, is responsible for the oxidation of tocopherol to tocopheryl-quinone. Controls containing different amounts of synthetic tocopherol, as well as extracts to which tocopherol had been added, when treated with carbon, were found to contain only 25% of the original tocopherol, the remaining part of it having been transformed to tocopheryl-quinone.

After absorption on carbon of the extract, a second substance, closely resembling a Ubiquinone homologue, can easily be detected. This is liquid at room temperature and, in the oxidized state, shows a typical band in the UV with a peak at 279 mμ. All the Ubiquinone homologues have in ethanol an UV band displaced to shorter wavelength (275 mμ). The carbon treatment does not seem to be responsible for the shifting of the Ubiquinone band from 275 to 279 mμ. Ubiquinone 50 and 30, after absorption on carbon and elution with chloroform, show no change in their spectrophotometric properties. The low melting point could suggest a very short chain or perhaps a modified structure of the compound, whereas the peak at 279 mμ may indicate the presence of hydroxyl group(s) in the side chain[15].

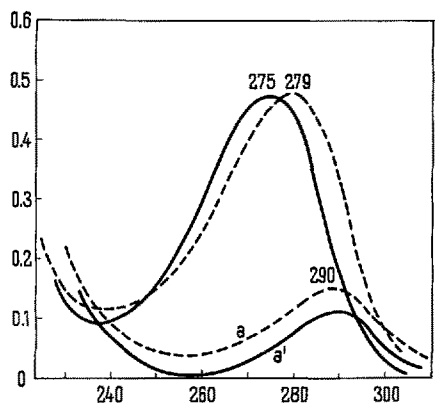

Fig. 2. Ultraviolet spectrum of fraction S_2 (---) as compared with Ubiquinone 50 prepared from beef heart (—). a and a', after reduction with KBH_4.

Riassunto. L'insaponificabile del muscolo di *Spirographis spallanzani* contiene accanto a steroli e tocoferolo, un composto di natura chinonica avente molte proprietà in comune con gli ubichinoni. Anche il tocoferilchinone è stato identificato negli estratti. Si ritiene però che esso si formi dal tocoferolo durante il procedimento di estrazione.

G. CASERTA, G. NARDI, and F. GHIRETTI

Reparto di Fisiologia e Biochimica, Stazione Zoologica, Napoli (Italy), January 31, 1964.

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The Optical Rotatory Dispersion Curves of *trans*-Decalones and their Polycyclic Analogues[1]

Work on optical rotatory dispersion curves over the last 10 years has produced a mass of data on ketones, the $n \rightarrow \pi^*$ carbonyl absorption band at 290 mμ being specially suitable for measurement. General reviews have been given[2-4], and the ideas on this subject have been systematized in terms of the Octant Rule[5]. Extensive collections of data for six-membered ring ketones have been given elsewhere[6,7].

It has been apparent for some time[8] that the amplitude (*a*) of the Cotton effect is a convenient measure of the asymmetry of the surroundings of the carbonyl group. (The amplitude *a* is defined as the molecular rotation at the extremum of longer wavelength *minus* the molecular rotation at the extremum of shorter wavelength; i.e. the difference between peak and trough, appropriately signed.) From the theoretical standpoint, the rotational

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