Table I. Incorporation of label from [1-^{14}C]-acetate into fatty acids and 19-nor-stanols by *A. polypoides*[a]

	Weight (g)		dpm/mg[b]	
	48 h	290 h	48 h	290 h
Lyophilized animals	80	75		
Fatty acid methyl esters	0.41	0.52	3,070	4,160
Crude stanol fraction	0.17	0.15	—	—
After recrystallization	0.1	0.09	134	132
After conversion to acetates and purification by chromatography and crystallization	0.06	0.06	59	67

[a] [1-^{14}C]-acetate (62 mCi/mmole; 0.25 mCi) was fed to the animals by addition of 5 ml aqueous solution to the aquarium (50 l). 48 h after the administration, ca. half of the animals were taken, washed and frozen at −20° and the remaining animals were killed after 290 h incubation
[b] The radioactivity was measured by a Beckmann LS-250 liquid scintillation system.

Acetate was readily incorporated into fatty acids, but utilized only to a very low extent for the biosynthesis of 19-nor-stanols (Table I), suggesting that there is little or no de novo sterol biosynthesis. In view of the same very low radioactivity recovered in the stanols from both 48 h and 290 h feeding experiments, we presume that it must be due to contamination and, accordingly, the sponge, under such conditions, is unable to synthesize sterols from acetate. When specimens of *A. polypoides* were incubated with [26-^{14}C]-cholesterol, radioactivity was efficiently incorporated into the 19-nor-stanols (Table II) and almost all of this radioactivity corresponded to the 19-nor-cholestanol fraction, as shown by preparative GLC of the derived phenyl acetates mixture and determination of the radioactivity of the resolved individual components (**9**–**11**)[8].

These results indicate that *A. polypoides* readily removes the 10-methyl group from cholesterol substrate and suggest that in the sponge these unique 19-nor-stanols arise by modification (removal of 10-methyl group) of dietary sterols[9].

Table II. Incorporation of [26-^{14}C]-cholesterol into 19-nor-stanols by *A. polypoides*[a]

Period of incubation	Lyophilized animals (g)	Total fed (dpm)	Total sterol recovered		Radioactivity recovered (%)	Radioactivity recovered (%)	
			(mg)	(dpm)		Precursor[b]	19-Nor-stanols[c]
48 h	38	5.5×10^8	220	2.03×10^6	0.37	80	20
290 h	25	5.5×10^8	305	11.3×10^6	2.04	22	78

[a] [26-^{14}C]-cholesterol (61 mCi/mmole) was fed to the animals by addition of 2 ml ethanolic solution to the aquarium (50 l). 48 h after the administration, ca. half of the animals were taken, washed with water and frozen at −20 °C, and the remaining animals were killed after 290 h incubation. [b] Radioactivity was measured on cholesta-1,4-dien-3-one (see text). [c] Radioactivity was measured on the derived phenols and phenyl acetates purified to constant specific activity (see text).

[8] Portion of the labelled phenyl acetates from 19-nor-stanols deriving from 48 h incubation with [26-^{14}C]-cholesterol was added to a mixture of carrier phenyl acetates to obtain a specific radioactivity of 4×10^2 dpm/mg and subjected to preparative GLC. A Carlo Erba gaschromatograph, model G-V, equipped with a flame ionization detector was used and the separation was performed using a 2-m glass column (i.d. 6 mm) packed with 1% OV-1 Gas-Chrom 80–100 mesh and operated at 260° with N_2 at 120 ml/min. The first peak corresponding to 19-norcholesta-1,3,5(10)-trien-3-yl acetate (**9**) (31.6% of the total mixture) has a specific radioactivity of 1.2×10^3 dpm/mg.

[9] Acknowledgments. We thank the Zoological Station (Naples) for provision of the sponges and use of Laboratory facilities. The technical assistance of Mr. A. Crispino is also acknowledged.

Riassunto. La spugna *Axinella polypoides* trasforma il [26-^{14}C]-colesterolo nel 19-nor-colestanolo (**1**), mentre non utilizza l'[1-^{14}C]-acetato per la sintesi dei 19-nor-stanoli (**1**–**8**). Si suggerisce che questi unici stanoli si originano da steroli dietarici per rimozione del metile in 10.

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Preparation of Synthetic Rotenoids

In a previous report, the synthesis of a new dehydrorotenoid was described by ringclosure of deoxybenzoine derivatives[1]. Now we wish to report the preparation of new rotenoids by thermal condensation of 4-ethoxycarbonyl-3-chromanones with *O*-heterocyclic phenols, affording dehydrorotenoids which were transformed into rotenoids by catalytic hydrogenation[2,3].

Heating of a mixture of 4-ethoxycarbonyl-3-chromanones (**1**) (0.15 mmol) with the phenols (**2**, **5**) (0.1 mmol) at 160–170 °C/12 mm Hg for 4 h gave the dehydrorotenoids (**3**, **6**), which were easily isolated from the reaction mixture by trituration with ether. When the reaction was carried out in boiling diphenyl ether, the yields were slightly decreased.

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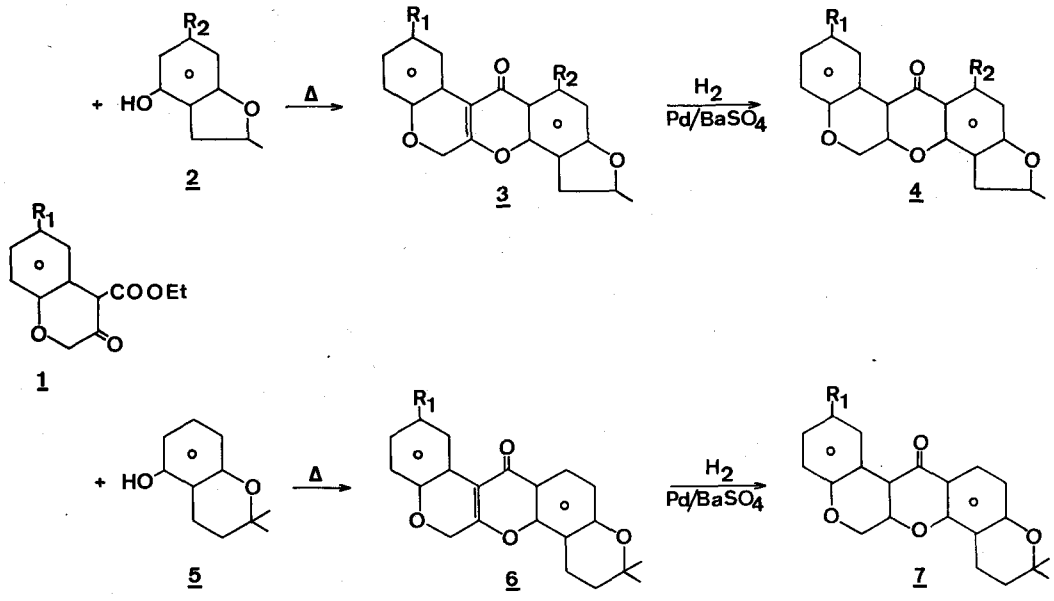
The IR-spectra of dehydrorotenoids displayed the typical pattern of a γ-pyron ring at 1650–1570 cm^{-1} (CO and C=C)[4]. The structure of **3** and **6** were confirmed by NMR and MS.

Hydrogenation of the dehydrorotenoids **3** (R_1=R_2=Me) and **6** (R_1=H) in a Parr apparatus with $Pd/BaSO_4$ in methanol at 4 atm. gave the rotenoids **4** (R_1=R_2=Me) and **7** (R_1=H). The rotenoid **4** (R_1=R_2=Me) was identical with a sample prepared by the MIYANO procedure[5], which consisted of the sodium borohydride reduction of the dehydrorotenoid **3** (R_1=R_2=Me) and successive Oppenauer oxidation.



Compound			Yield (%)	M.p.	IR (KBr) cm^{-1}
	R_1	R_2			
3	H	H	40	>300°	1635, 1605, 1560
3	Me	H	33	243°	1642, 1610
3	Me	Me	49	227°	1645, 1620, 1590
6	H	—	37	219°	1635, 1600, 1580, 1570
4	Me	Me	90	153°	1670, 1590
7	H	—	79	181°	1670, 1600

The IR of the rotenoids **4** and **7** now exhibited the carbonyl function at 1670 cm^{-1}, while the stereochemistry and the conformation was established by NMR confirming the *cis*-B/C fusion alloted to natural rotenoids[6].

Zusammenfassung. Eine einfache Synthese von Rotenoiden durch Kondensation von 4-Ethoxycarbonyl-3-chromanonen mit Phenolen und katalytischer Hydrierung der entstehenden Dehydrorotenoide wird beschrieben.

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Ultrastructural and Physico-Chemical Properties of a Polymeric Macromolecular Serum Protein

Much information has accumulated during the last decade concerning the fine architecture of many plasma proteins. Certain plasmaproteins, e.g. immunoglobulins and isoenzymes, can occur in polymeric form. We report here on the ultrastructure of a polymeric macromolecular protein population in human serum, the fine architecture of which has not been described before.

Materials and methods. Human serum was fractionated, starting with the removal of low density lipoproteins[1]. To the supernatant was added $(NH_4)_2SO_4$ solution, pH 7.2 (final concentration 1.5 *M*). The dissolved precipitate was filtered on a Bio-Rad A15 column and fractions eluting before or in the IgM region (Figure 1) were found to contain the macromolecules when examined by electron microscopy (EM). These fractions were concentrated, dialyzed and subjected to Pevicon electrophoresis. 3 fractions in the (α_1-) post albumin region were pooled and concentrated (Figure 2). Low-weight molecular contamination was eliminated on a Sephadex G200 column. The concentrated macromolecular fractions (0.2 mg protein/ml) were tested by immunodiffusion[2] against rabbit antiserum to whole human serum, C1, C3, C4, IgG, IgA,

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