A NEW UBIQUITOUS COMPOUND STRUCTURALLY RELATED TO LIPID-QUINONES

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The presence of a compound exhibiting ultraviolet absorption peaks at 272 and 310 mμ was first reported in egg yolk and chick embryo (Ramasarma *et al.*, 1961) and since then it was detected in this laboratory in several natural sources: plants (*Tecoma stans* leaves, *Phaseolus radiatus* seedlings), moulds (*Aspergillus niger*, *Aspergillus fumigatus*), bacteria (*Escherichia coli*), yeasts (*Torula utilis*, a commercial dried yeast), a snail (*Cryptozona semirugata*), a protozoan (*Blepherisma undulans*) and rat liver. It is synthesized, along with nonaprenyl phenol, from phenol and solanesol in presence of borontrifluoride as the catalyst suggesting thereby that this was a polyprenyl phenolic compound. The synthetic and natural compounds had identical ultraviolet absorption spectra, showed highly non-polar elution characteristics and same R_F values in adsorbant chromatography and gave positive test with the phenolic spray (diazo-sulfanilic acid). It is proposed to refer to this as " Compound S " until its chemical structure is established and a suitable name assigned.

A typical method of preparation using Tecoma stans leaves consisted of saponifying the dry powder (100 g) in methanol and fractionating the unsaponifiable lipids on 5% deactivated alumina column (50 g) with the following eluants: light petroleum (500 ml), 20% benzene/light petroleum (250 ml) and 5% ether/light petroleum (250 ml). " Compound S " was eluted in 20% benzene and ubiquinone in 5% ether. Fractions containing " Compound S " were rechromatographed on thin layer plates of silica gel-G with 50% benzene/light petroleum as the developing solvent and the pure compound (yield, 2.0 mg) was isolated from the appropriate area (R_F, 0.54). The procedure was essentially the same for other sources.

The compound was chemically synthesized from phenol (20 mg) and solanesol (50 mg) in dioxane (20 ml) in presence of borontrifluoride (1.0 ml) by incubating the mixture for 2 hr at room temperature. The isolation method was the same as described above (yield, 1.9 mg). Nonaprenyl phenol and another more polar phenolic lipid were also synthesized under these conditions.

" Compound S " showed blue fluorescence on exposure to ultraviolet light. It gave stable pink color on spraying with a solution of diazo-sulfanilic acid in alkali (Trutter, 1963), in contrast to yellow to brown color obtained with phenolic compounds (and also by 6-methoxy-2:2-dimethyl chromene). The ultraviolet absorption spectrum showed two peaks at 272 and 310 mμ ($E^{1\%}_{1cm}$ values: 72 and 46 for natural compound from T. stans and 60 and 44 for synthetic compound, respectively) with shoulders at 260 and 298 mμ (Fig. 1).

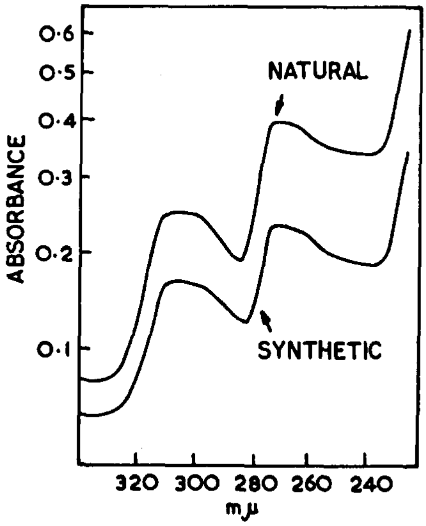

Fig. 1: Ultraviolet absorption spectrum of " Compound S " Natural, from T. stans (0.054 mg/ml); Synthetic (0.038 mg/ml). The spectra were taken in a Beckman Model DB Recording spectrophotometer.

The spectrum was unaltered on adding sodium borohydride. Treatment with silver oxide resulted in the loss of the characteristic spectrum, the fluorescence and the pink color in the phenolic test. The spectrum did not show any shifts in the presence of alkali indicating the absence of free phenolic group. Catalytic hydrogenation in ethanol with palladium on charcoal as catalyst yielded a product having a single absorption peak at 265 mμ. These characteristics are typical of styrene structure and closely resemble those of ubichromenol (Laidman *et al.*, 1960), plastochromenol (Rowland, 1958), Ageratochromene (Alertsen, 1955), 6-methoxy-2:2 dimethyl chromene (Kasturi and Thomas, 1967) and 1:2 chromene (Chatterjea, 1959). Infrared spectrum of " Compound S " showed major bands at 800, 1020, 1100, 1270, 1470, 1640, 2850, 2925 and 2960 cm^{-1} consistent with chromene and isoprene structures. The region of 3300-3600 had no band showing the absence of free hydroxy group. Mobility of " Compound S " in various chromatographic systems revealed its non-polar nature, relative to ubiquinone and nonaprenyl phenol, thereby supporting that the oxygen function was in bound form and not as a free phenol (Table 1). The above data support the following structural features of " Compound S," consistent with the structure of polyprenyl chromene.

1. polyprenyl phenolic compound without substitutions on the ring as required from the synthetic data.
2. the oxygen function is not free phenolic as supported by the absence of -OH stretching in the infrared spectrum, lack of change in ultraviolet spectrum on addition of alkali and the non-polar behaviour in adsorbant chromatography.
3. possession of styrene-type ultraviolet spectrum with twin peaks changing to one on catalytic hydrogenation.

Multiprenyl phenol was shown to be the precursor of ubiquinone in the anaerobic synthesis of *Rhodospirillum rubrum* (Olsen *et al.*, 1966). It was not possible to detect prenyl phenols in three species synthesizing ubiquinone aerobically, the rat, *T. stans* and *A. niger*; instead " Compound S " was consistently found in a number of aerobic organisms. The presence of " Compound S " was not due to any artifact formation during saponification since it could be detected in total lipids obtained without saponification. It is conjectured that " Compound S " is potentially significant in the

Table 1. R_F values of " Compound S "

	Thin layer			Reverse phase					
	Silica gel-g		Alumina	Silicone fluid 220			Vaseline		
	I	II	III	IV	V	VI	IV	V	VI
" Compound S " synthetic	0.82	0.54	0.70	0.83	0.52	0.68	0.78	0.60	0.75
natural from *T. stans*	0.82	0.54	0.70	0.83	0.52	0.68	0.78	0.60	0.75
* Nonaprenyl phenol	0.76	0.36	0.18	0.19	0	0	0.12	0	0
Ubiquinone - 9	0.32	0.14	0.23	0	0	0	0	0	0
Ubichromenol - 8	0.25	0	0.06	0	0	0	0	0	0
Selanesol	0.20	0	0	0	0	0	0	0	0

I. Benzene; II. 50% Benzene/light petroleum; III. 20% Benzene/light petroleum; IV. 60% n-Propanol/water; V. 40% n-Propanol/water; VI. 80% Methanol/aqueous ammonium acetate (1 M). (* Relative to ubiquinone, nonaprenyl phenol behaves more polar on alumina and less polar on silica gel).

aerobic synthesis of lipid-quinones and related compounds (ubiquinone, ubichromenol, plastoquinone, tocopherol and vitamin K).

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