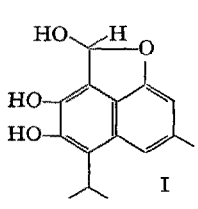
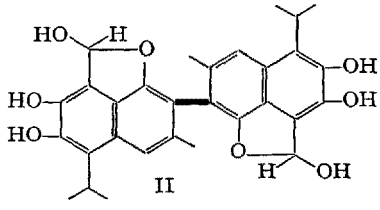
thespesin and more particularly those of its derivatives. These spectra are sharply defined and the proton count of any peak can be halved. The NMR-spectrum of thespesin, determined in carbon tetrachloride, has a doublet at 8.60 τ (12H) and a singlet at 7.92 τ (6H) indicating the presence of 2 isopropyl groups (supported by $\nu_{max}^{CHCl_3}$ 1375, 1360, 1170 and 1128 cm^{-1}) and 2 aromatic methyl groups respectively. Other features are 3 singlets for hydroxylic protons at 4.10 τ (2H), 2.8 τ (2H) and $-$ 4.4 τ (2H), an aromatic proton singlet at 2.42 τ (2H) and a benzylic proton multiplet centred at 6.25 τ (2H). Its IR-spectrum indicates the presence of bonded and non-bonded phenolic hydroxyls in the molecule ($\nu_{max}^{CHCl_3}$ 3350, 3300, 3500 and 1195 cm^{-1}) and its aromatic nature ($\nu_{max}^{CHCl_3}$ 3040, 1621, 1600 and 1575 cm^{-1}). The NMR-spectrum of thespesin hexamethyl ether in carbon tetrachloride has a doublet for 2 isopropyl groups (8.52 τ), a singlet for 2 aromatic methyl groups (7.88 τ), a singlet for 2 aromatic protons (2.30 τ) and a multiplet for 2 benzylic protons centred at 6.25 τ. The protons of the 6 methoxyl groups appear as 3 singlets at 6.12 τ (6H), 6.20 τ (6H) and a highly shielded 6.88 τ (6H). Hexaacetyl thespesin, $C_{42}H_{42}O_{14}$ (M^+ 770), m.p. 186–188 °C, $[\alpha]_D$ + 328° (benzene), prepared by treatment of thespesin with acetic anhydride-sodium acetate, had a similar NMR-spectrum but its IR-spectrum had no aldehyde C=O absorption.

The molecular ion peak (M^+ 518) in the mass spectrum of thespesin is weak. The cracking pattern is characterized by the loss of 2 molecules of water, $-H_2O$ (m/e 500) and $-2H_2O$ (m/e 482, base peak) and the subsequent loss of other functional groups: $-CH_3$ (m/e 467), $-C{=}O$ (m/e 454), $-CHO$ (m/e 453), $-(CH_3 + C{=}O)$ (m/e 439), $-CH(CH_3)_2$ (m/e 439) and $-CH_3-\overset{+}{C}H{=}CH_2$ (m/e 441). The other feature of this spectrum is the abundance of doubly charged ions m/e 241 downwards, and is good evidence for the aromatic nature of thespesin and the dimeric nature of the molecule. The mass spectrum of hexaacetyl thespesin shows similar fragmentation but the base peak in the mass spectrum of hexamethyl thespesin is the molecular ion peak (M^+ 602).

On the basis of the above data, thespesin is a 1,1′ or a 2,2′ dimer of the sesquiterpenoid naphthalene I. Gossypol[3], the well-known pigment from cotton seed, has the structure II but is optically inactive and so far has only been isolated from Gossypium species. The UV-spectrum of thespesin and gossypol (λ_{max}^{EtOH} 236 nm (77,300), 280 nm (shoulder), 289 nm (30,740) and 373 nm (16,800)) were determined under identical conditions and found to be superimposable as were also their IR-spectra. The UV-spectra of 1,1′ and 2,2′ binaphthalenes are distinguishable[4] (extension of transverse and longitudinal polarized bands respectively). Thespesin must therefore be an optically active (+)-isomer of gossypol, now encountered for the first time.



I



II

Treatment of thespesin with acid does not alter its optical rotation. It would therefore appear that the asymmetric hemiacetyl carbon atoms make no contribution and that the optical activity of thespesin is due to restricted rotation of the 2 naphthalene units about the interlinking C–C bond (atropisomerism). Atropisomerism has recently been observed in (–)-isodiospyrin[5], a binaphthaquinone isolated from *Diospyros chloroxylon*[6].

Zusammenfassung. Thespesin, der optisch aktive gelbe Farbstoff aus den Früchten der *Thespesia populens* Soland hat eine dimere sesquiterpene-naphthalene Struktur, die mit dem optischen inaktiven Baumwollsamen-Farbstoff Gossypol identisch ist. Die optische Aktivität des Thespesin ist bedingt durch Atropisomerie.

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Distribution of Hydroxyproline and Hydroxylysine Deficient Collagen in Individual Collagen Fractions in the Granuloma Tissue

UDENFRIEND's[1] and PROCKOP's[2] laboratory established evidence that formation of non-hydroxylated, proline-rich collagen polypeptide is one of the steps leading to the synthesis of collagen molecule. There is sufficient proof of the formation of hydroxyproline and hydroxylysine deficient collagen under rather artificial conditions, such as blocking of ferrous ions by chelating agents[3], ascorbic acid deficiency[4], low oxygen tensions[5] or nitrogen atmosphere[6].

Recently, JUVA et al.[7] submitted very convincing autoradiographic evidence that in the presence of the hydroxylation blocking agents (2,2′-dipyridyl or nitrogen atmosphere) proline-H[3] (which probably corresponds to protocollagen) accumulates over the cells of incubated cartilage slices. In control samples, the isotope was uniformly distributed in the tissue. The authors conclude that collagen hydroxylation is the essential step for utilization of polypeptide chains in the formation of collagenous triple helix and its secretion into the extracellular space. Since under the conditions mentioned synthesis and secretion of mucopolysaccharides was not influenced, the authors assume that dipyridyl would not block reactions connected with

the transfer of collagen molecule from intracellular into extracellular space.

This conclusion was contradictory to our findings. We isolated in experiments with chick embryo skin slices C^{14} labelled atypical collagen also in the fraction of less soluble collagens which were represented by the residue left after 0.2 *M* NaCl extraction[3,5]. That was the reason why we checked the experiments by JUVA et al.[7].

Granulation tissue was isolated on the seventh day after s.c. injection of 5 ml of 1% solution of carrageenan in guinea-pigs. Five g of the minced tissue was incubated in 17 ml of Krebs-Ringer bicarbonate buffer with 8.92 μC proline-C^{14}. The incubation lasted 2 h at 37 °C in the atmosphere 95% O_2 + 5% CO_2. Experimental samples contained 2,2'-dipyridyl in 0.5 m*M* concentration. From the total of 30 g of homogenized tissue incubated without and with dipyridyl, 3 types of collagen proteins were isolated. Neutral salt soluble collagens (NSC) were extracted twice each for 24 h at 4 °C with 0.2 *M* NaCl, pH 7.4 containing 1 m*M* sodium EDTA. From the pooled extracts all proteins were precipitated with cold trichloroacetic acid (TCA) in final concentration of 15%. Collagen proteins were reextracted from the sediment according to FITCH et al.[8]. TCA extracts were dialysed for 16 h against tap water. From the residue, acid soluble collagens (ASC) were isolated by extraction into 0.1 *M* acetic acid by the same procedure as NSC. Insoluble collagen (ISC) was extracted from the residue into hot 0.3 *M* TCA according to FITCH et al.[8]. Vacuum dried extracts were hydrolysed (6 *N* HCl, 105 °C, 16 h). The separation of proline and hydroxyproline was carried out using descending paper chromatography with repeated development on Whatman No. 3 in a mixture buthanol-acetic acid-water (4:1:5). After elution of corresponding spots the amount of proline[9] and hydroxyproline[10] was determined. Radioactivity assays were performed on Packard-Tri-Carb-Liquid Scintillation Spectrometer Model 3365 with 61% efficiency in a mixture of 4.0 ml absolute ethanol, 5.0 ml scintillation solution (4 g of 2,5-diphenyloxazole and 100 mg of 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene in 1000 ml toluene) and 0.4 ml sample.

The results summed up in the Table show that under dipyridyl effect the hydroxylation of collagenous proline was inhibited and that this atypical collagen was present in all 3 fractions of collagen proteins investigated.

This finding therefore contradicts the belief that atypical collagens cannot aggregate and mature. The presence of hydroxyproline deficient collagen in the fraction of insoluble collagen shows that it is released by the fibroblasts into extracellular space. Recently KIVIRIKKO and PROCKOP[11], characterizing the size of collagen formed in embryonic cartilage under the effect of 2,2-dipyridyl, arrived at the conclusion that most of the collagen polypeptides accumulated are as large as complete α-chains of collagen. The reason for this difference in results might be the fact that we used another tissue as a biological substrate. We found namely in our preliminary autoradiographic studies differing susceptibility and reactivity of various tissues towards the action of some chelating agents[12].

The accumulation of protocollagen formed under the nitrogen atmosphere over the cells could be explained by experiments of TSURUFUJI and OGATA[13]. The maturation of neutral salt soluble collagen to insoluble collagen was interrupted by replacing the oxygen by nitrogen in the gas phase. Our own data show also a close relation between the maturation of soluble collagen and oxygen tensions[5].

When comparing the magnitudes of specific activities of hydroxyproline-C^{14} in individual collagens we get the order NSC > ISC > ASC which agrees with the finding of KÜHN et al.[14] and TSURUFUJI and OGATA[13]. It seems that ASC is rather a degradation product of ISC than its precursor.

Distribution of the label in different fractions of collagen from granuloma tissue

Sample	Amount			Specific activity	
	hyp μg	pro μg	pro/hyp	hyp 10^{-2} cpm per μmole	pro
NSC control	278	510	1.89	18.0	28.1
dipyridyl	225	439	1.96	3.7	74.6
ASC control	189	302	1.61	3.3	7.6
dipyridyl	280	368	1.31	1.2	19.5
ISC control	1680	1960	1.16	8.0	10.6
dipyridyl	1880	2340	1:24	1.7	19.5

For isolation 30 g of tissue (wet weight) were used. Hyp, hydroxyproline; pro, proline.

Zusammenfassung. Vom atypischen C^{14}-Kollagen, das unter der Wirkung von 0.5 m*M* 2,2-Dipyridyl im karrageenen Granulationsgewebe entstanden ist, wurden neutralsalzlösliche, säurelösliche und unlösliche Fraktionen isoliert. Die Erscheinung der Radioaktivität in der unlöslichen Fraktion beweist, dass atypisches Kollagen von Zellen transportiert wird und im extrazellulären Raum aggregieren kann. Die niedrigere spezifische Aktivität des säurelöslichen Kollagens zeigt, dass es sich um ein Abbauprodukt des unlöslichen Kollagens handelt.

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