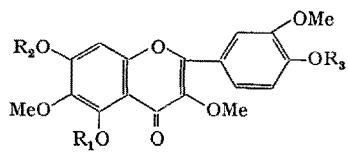
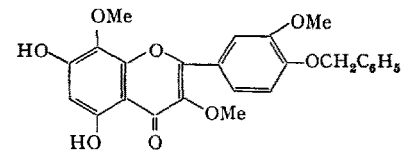
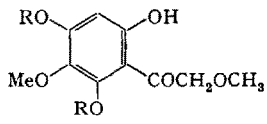


I $R_1 = R_3 = H$, R_2 = Glycosyl
II $R_1 = R_2 = R_3 = H$
V $R_1 = R_2 = H$, $R_3 = CH_2C_6H_5$
VIII $R_1 = R_2 = Et$, $R_3 = CH_2C_6H_5$
IX $R_1 = R_2 = R_3 = CH_3CO$
X $R_1 = R_2 = R_3 = Me$
XI $R_1 = R_2 = R_3 = Et$



VI



III R = H
VII R = Et

obtained with diethyl sulphate, was prepared from 3,5-diethoxy-4-methoxyphenol by an unambiguous method.

By a similar Hoesch condensation the phenol gave 4,6-diethoxy-2-hydroxy-5,ω-dimethoxyacetophenone (VII) (b.p. 145–146 °C/0.2 mm. Found: C, 59.24; H, 7.37. $C_{14}H_{20}O_6$ requires: C, 59.14; H, 7.09%). According to the ALLAN-ROBINSON's flavone synthesis, the ketone (VII) with IV yielded 4'-benzyloxy-5,7-diethoxy-3,6,3'-trimethoxyflavone (VIII) (m.p. 117–118 °C, IR 1638 cm^{-1} (γ-pyrone) (Nujol), UV λ_{max}^{EtOH} nm (log ε): 336 (4.27). Found: C, 69.03; H, 6.04. $C_{29}H_{30}O_8$ requires: C, 68.76; H, 5.97%). This compound was identified by direct comparison with the diethyl compound, which was obtained by the ethylation of the hydroxyflavone. From this fact, the structure of the hydroxyflavone was established as 4'-benzyloxy-5,7-dihydroxy-3,6,3'-trimethoxyflavone (V). Then, the debenzylation of V with hydrogen afforded the desired flavone (II) (m.p. 130–135 °C (from methanol-water). Found: C, 58.57; H, 4.82. $C_{18}H_{16}O_8 \cdot {}^1/_2H_2O$ requires: C, 58.54; H, 4.64%. After drying 50–60 °C/10^{-1} mm for 2 h, m.p. 166.0–166.5 °C (127 °C sinter). Found: C, 59.86; H, 4.48. $C_{18}H_{16}O_8$ requires: C, 60.00; H, 4.48%. IR 3560, 3250 (OH), 1655 cm^{-1} (γ-pyrone) (Nujol), UV λ_{max}^{EtOH} nm (log ε): 257 (4.18), 272.5 (4.20), 355 (4.28) (lit.[1] m.p. 127–133 °C, UV λ_{max}^{MeOH} nm (log ε): 255 (4.23), 350 (4.32) (lit.[2] m.p. 165–166 °C, UV λ_{max}^{EtOH} nm (log ε): 255 (4.25), 270 (4.19), 354 (4.35), whose identity with natural jaceidin[4] was confirmed by mixed m.p. determination, IR- and UV-spectral comparison. Its triacetate (IX) (m.p. 163–164 °C) (lit.[2] m.p. 159–160 °C), trimethyl ether (X) (m.p. 142–143 °C) (lit.[2] m.p. 142–143 °C) and triethyl ether (XI) (m.p. 118–119 °C (lit.[1] m.p. 118 °C) were prepared by a usual method. Also, XI could be derived from VII and *O*-ethylvanillic anhydride by a similar method.

Zusammenfassung. Durch Kondensation nach ALLAN-ROBINSON wurde aus 2,4,6-Trihydroxy-3,ω-dimethoxyacetophenon das 4'-Benzyloxy-5,7-dihydroxy-3,6,3'-trimethoxyflavon dargestellt. Entbenzylierung in C-4'-Stellung führte zum 5,7,4'-Trihydroxy-3,6,3'-trimethoxyflavon. Die Eigenschaften dieser Verbindung und ihre Derivate sind identisch mit dem aus *Centaurea jacea* L. isolierten Jaceidin bzw. seinen Derivaten.

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[4] We are grateful to Prof. H. WAGNER, University of München, for his gifts of natural jacein and jaceidin.

The Chemistry of Thespesin[1]

Thespesin[2], a yellow crystalline compound, m.p. 196 to 197 °C, $[\alpha]_D$ + 457° (benzene), isolated from the fruit of *Thespesia populens* Soland, has now been assigned the molecular formula $C_{30}H_{30}O_8$ on the basis of its mass spectrum (M^+ 518).

Three interesting features of the chemistry of thespesin are the presence of potential aldehyde functions in the molecule, the apparent symmetry of the molecule, and its strong dextrorotation. The IR-spectrum of thespesin is devoid of any C=O absorption between 1625 and 1800 cm^{-1}, but thespesin readily forms a dioxime m.p. 320 °C, a dianilino derivative m.p. 305 °C and a di-2,4-dinitrophenyl hydrazone m.p. 215–220 °C. On methylation in acetone with dimethyl sulphate in the presence of potassium carbonate, a colourless hexamethyl ether $C_{36}H_{42}O_8$ (M^+ 602), m.p. 242–244 °C, $[\alpha]_D$ + 177° (chloroform) is formed. This compound has a discernible aldehyde carbonyl function (ν_{max}^{KBr} 1690 cm^{-1}) and forms the expected dioxime m.p. 200–204 °C and a diphenyl hydrazone m.p. 266–268 °C. These observations can be explained if the thespesin molecule has 2 hemiacetyl functions.

The apparent symmetry of the thespesin molecule is indicated by the uniqueness of the NMR-spectrum of

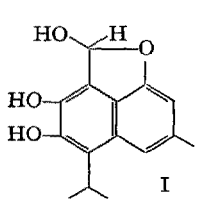
[1] Communication No. 1225 from the Central Drug Research Institute.

[2] S. N. SRIVASTAVA, D. S. BHAKUNI and V. N. SHARMA, Indian J. Chem. *7*, 451 (1963).

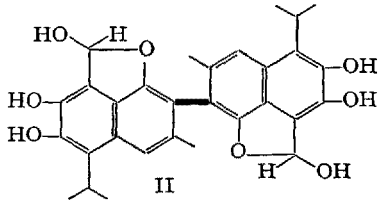
thespesin and more particularly those of its derivatives. These spectra are sharply defined and the proton count of any peak can be halved. The NMR-spectrum of thespesin, determined in carbon tetrachloride, has a doublet at 8.60 τ (12H) and a singlet at 7.92 τ (6H) indicating the presence of 2 isopropyl 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.

D. S. BHAKUNI, M. M. DHAR and V. N. SHARMA

Central Drug Research Institute and National Botanical Gardens, Lucknow (India), 26 October 1967.

[3] R. ADAMS, T. A. GEISSMAN and J. D. EDWARDS, Chem. Rev. *60*, 555 (1960).
[4] H. H. JAFFE and M. ORCHIN, *Theory and Application of Ultra-Videt Spectroscopy* (John Wiley & Sons, New York 1962), p. 302.
[5] G. S. SIDHU and K. K. PRASAD, Tetrahedron Lett. 2905 (1967).
[6] We thank Dr. R. S. KAPIL for mass and NMR-spectra and valuable suggestions.

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