

organo-borane oxidised by alkaline hydrogen peroxide, a complex mixture was obtained which on chromatography over alumina gave (\pm) O-trimethylbrazilin (III), m.p. 135–36° (acetate, m.p. 185–86°), an unidentified product, m.p. 285° and a considerable amount of a gummy product which was not examined further. The synthetic product was identical with an authentic sample obtained through the courtesy of Professor O. Dann^{3,4}. This synthesis is stereo-specific and proves that the ring junction of the chroman ring and the indane ring is *cis* in brazilin. When (III) was methylated by methyl iodide in presence of potassium hydroxide under carefully controlled condition (\pm) O-tetramethylbrazilin (IV), m.p. 113–14°, was obtained having superimposable i.r. spectrum in chloroform solution with natural (+) O-tetramethylbrazilin, m.p. 139–40°. It may be pointed out that this racemic compound is claimed to have been synthesised by Chakravarty⁵ who reported an m.p. 133–35° but there is little doubt Chakravarty's product must have a different structure.

Chemical Laboratory,
Patna University,
Patna-5.

J. N. CHATTERJEA.
S. C. SHAW.
N. D. SINHA.

October 24, 1972.

1. Perkin, W. H., Ray, J. N. and Robinson, R., *J. Chem. Soc.*, 1928, p. 1504.
2. Pfeiffer, P. and Co-workers, *Ber.*, 1927, 60, 2142; *Ibid.*, 1928, 61, 294, 839, 1923.
3. Dann, O. and Hofmann, H., *Liebigs Annalen*, 1963, 667, 116.
4. A synthesis of (+) brazilin was reported by Morsingh, K. and Robinson, R., *Tetrahedron*, 1970, 26, 281.
5. Chakravarty, P. M., *Tetrahedron Letters*, 1963, 27, 1907.

ISOLATION OF PONGACHALCONE-I FROM THE HEART-WOOD OF *PONGAMIA GLABRA* L.; MERR.

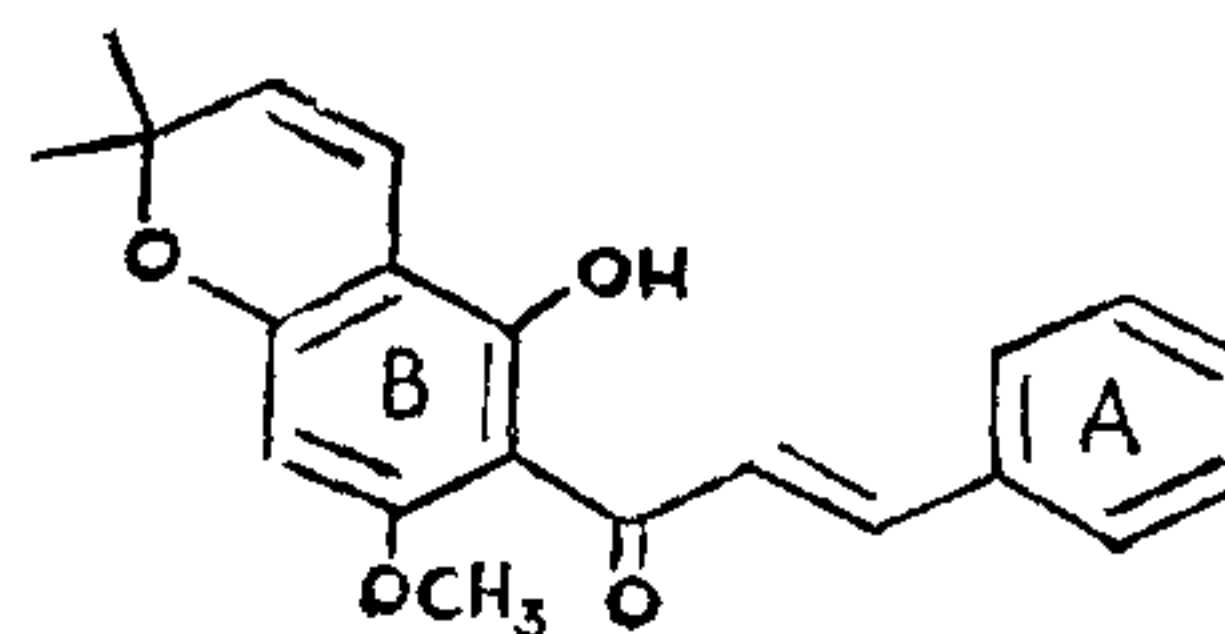
THE heart-wood of *Pongamia glabra* (fam. Leguminosae) has not been chemically examined so far, even though most other parts of the tree¹⁻⁵ have been. The hexane extract of the heart-wood, when subjected to thin layer chromatography on silica gel, showed as many as twenty-two distinct but closely moving spots. The concentrated hexane extract was chromatographed on a column of neutral alumina using hexane (fraction-1) and hexane with increasing ratios of benzene (4:1, fraction-2; 3:1, fraction-3; 1:1, fraction-4; 1:2, fraction-5) as eluants.

The hexane eluant (fraction-1) was found to contain waxy material only. The hexane-benzene (4:1, fraction-2) gave a solid which crystallised

from methanol as shining needles with m.p. 138°. It gave positive Liebermann-Burchard test. Its acetate melted at 134°. The compound was identified as β -sitosterol by comparing with an authentic sample.

The hexane-benzene (3:1, fraction-3) on concentration gave a deep red oily residue. It showed two orange red spots besides a number of spots as minor constituents when subjected to TLC on silica gel plates using benzene as developing solvent. It was subjected to preparative TLC on silica gel using benzene as solvent. Two compounds separated as two orange red bands. The compound from the upper band crystallized from hexane as bright red needles. It was named Pongachalcone-I. From the lower band was obtained the second red compound.

Pongachalcone-I has m.p. 108° (Found C, 75.21; H, 6.13; OCH₃, 9.45; C₂₁H₂₀O₄ requires C, 74.98; H, 5.95; OCH₃, 9.5%), R_f 0.86. It gave characteristic test for chalcones with SbCl₃ in CCl₄ producing a deep red copious precipitate. λ_{max} 358 nm (4.31), 306 nm (4.24) and 238 nm (4.19); peak at 358 nm shifted to 398 nm in the presence of AlCl₃ indicating the presence of chelated hydroxyl group. $\nu_{max}^{CHCl_3}$ 3575 (broad band, chelated hydroxyl), 1640 (chelated carbonyl), 1340 cm⁻¹ (gem-dimethyl). Alkaline permanganate oxidation of the compound yielded benzoic acid. The 100 MHz NMR spectrum showed signals at τ 8.55 [6 H, singlet C(CH₃)₂], τ 6.05 (3 H, singlet, OCH₃), τ 4.55 (1 H, doublet, J = 10 Hz), τ 3.35 (1 H, doublet, J = 10 Hz) (chromene ring double bond protons). Mass spectrum of Pongachalcone-I showed a molecular ion m/e 336 and other fragment ions at m/e 321 (M-15), m/e 259 (M-77), m/e 232 (M-104), m/e 217 (M-15-104), m/e 244 (M-15-17), m/e 131 and m/e 103. The peaks at m/e (M-77) and m/e 232 (M-104) in its mass spectrum are due to the loss of a phenyl group and a neutral styrene molecule indicating that the ring A is unsubstituted. From the above results Pongachalcone-I can be assigned structure I or its linear isomer.



Structure (1)

The aromatic proton in ring B is assigned the position para to the phenolic hydroxyl by the positive Gibb's test and hence the methoxyl will be meta to the hydroxyl. This suggests that the structure of Pongachalcone-I as 2'-hydroxy-6'-methoxy-6'', 6''-dimethyl pyrano (3'' : 2'' ; 3' : 4') chalcone (I).

To compare with a synthetic sample, isoevodionol was prepared according to the procedure described by Seshadri *et al.*⁷ and it was subjected to chalcone condensation with benzaldehyde. The product was obtained in very small yield. On comparison with natural sample on silica gel plate showed a yellow spot having the same R_f value as the natural compound.

The red compound from the lower band from TLC crystallised from benzene-hexane as deep red prisms. It has m.p. 126° (Found C, 69.37; H, 5.17; OCH₃, 8.20; C₂₂H₂₀O₆ requires C, 69.46; H, 5.30; OCH₃, 8.16%). It produced green colour with gallic acid and sulphuric acid indicating the presence of a methylenedioxy group. This was confirmed by the formation of piperonylic acid in permanganate oxidation. Its UV, IR, NMR and Mass spectral data are quite in agreement with those described for glabrachromene reported recently by Seshadri *et al.*⁸.

The benzene-hexane (1:1, fraction 4) was concentrated and subjected to preparative thin layer chromatography using ethyl acetate-benzene (1:4) as developing solvent. The main fluorescent band (UV) was collected and extracted with hot ethyl acetate. The solution was concentrated and hexane added. Fine woolly needles separated out, m.p. 144°. It gave positive tests for flavone and for the presence of methylenedioxy group. Mixed melting point with authentic desmethoxy kanugin³ was undepressed.

The hexane-benzene (1:2, fraction 5), when subjected to preparative TLC using ethyl acetate-benzene (1:4) as developing solvent, gave three more compounds in small quantities. They were further purified and identified to be Kanugin³, Pongaglabrone² and Pongachromene⁶ by their melting points, and UV, IR and NMR spectra.

Dept. of Chemistry, K. SUBRAHMANYAM.
Andhra University J. MADHUSUDHANA RAO.
Postgraduate Centre, K. V. JAGANNADHA RAO.
Guntur-5, Andhra Pradesh,
October 7, 1972.

1. Aneja, R., Khanna, R. N. and Seshadri, T. R., *J. Chem. Soc.*, 1963, p. 163.
2. Khanna, R. N. and Seshadri, T. R., *Tetrahedron*, 1963, , 219.

3. Mittal, O. P. and Seshadri, T. R., *J. Chem. Soc.*, 1956, p. 2176.
4. Rangaswami, S., *Curr. Sci.*, 1946, 15 A, 127.
5. Murti, P. B. R. and Seshadri, T. R., *Proc. Ind. Acad. Sci.*, 1944, 20 A, 279.
6. Mukerjee, S. K., Sarkar, S. C. and Seshadri, T. R., *Tetrahedron*, 1969, 25, 1063.
7. Bajwa, B. S., Pyare Lal, R. and Seshadri, T. R., *Indian J. Chem.*, 1971, 9, 17.
8. Sulekha Mahey, Pushpa Sharma, Seshadri, T. R. and Mukerjee, S. K., *Ibid.*, 1972, 10, 585.

ANTIVIRAL SPECTRUM OF A GROWTH PRODUCT DESIGNATED 6-MFA ISOLATED FROM *ASPERGILLUS FLAVUS* L.

WE reported earlier that fungus *Aspergillus flavus* strain 6-MFA produced an anti-Semliki¹ Forest virus (SFV) substance(s) (designated 6-MFA) during growth in liquid culture.

We have now extended our observations to 3 more viruses, namely, Chikungunya (VRC M 42360-69, neurovaccinia, IHD strain, and dengue Type 2 (VRC TR 1751-M 27582), which are reported here.

Swiss CDRI mice, 35 days old, and body weight 16-18 gm were used in the present work. Semliki Forest virus (original strain) and neurovaccinia, IHD strain were obtained from ATCC, USA and maintained in Swiss mice. The other two viruses, *i.e.*, Chikungunya and dengue Type 2 were obtained as frozen dried ampoules from Virus Research Centre, Poona, India. *Aspergillus flavus* strain 6-MFA was grown in stationary culture in 1 litre conical flasks with 150-200 ml of glucose-nitrate-yeast medium² at 26° ± 1° C for 5-7 days. The mycelial mats were pooled and mashed in the same culture filtrate in a homogenizer (Mixi, Bajaj). The mashed suspension was centrifuged and debris discarded. To the clarified extract an equal volume of chilled acetone (BDH) was added. The precipitate (crude 6-MFA) was collected by centrifugation and dissolved in a volume of distilled water equivalent to one-half of the original crude filtrate. The aqueous preparation of 6-MFA was frozen dried. Mice were injected with 6-MFA at the rate of 3 mg intraperitoneally (i/p) and challenged 24 hrs later by SFV (100 LD₅₀, 0.5 ml, mouse) by the subcutaneous route (s/c), and by intracerebral route (i/c) with the other three viruses. Animals were observed morning and evening for 14 days for specific mortality.

Results are presented in Table I. It was seen that with the dose of SFV that killed 100% of the untreated control mice, 6-MFA protected 100% of the infected mice. In the case of Chikungunya virus (10 LD₅₀, *i.e.* 0.03 ml.) 6-MFA protected only