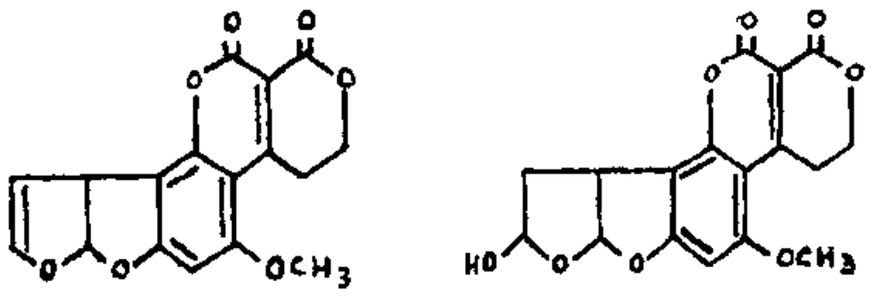
on the latter. In view of this, the present investigation was undertaken to prepare aflatoxin G_1 hemiacetal by direct hydration of aflatoxin G_1 (I) and to prove its identity with aflatoxin G_{2a} (II).



I

II

The hemiacetal is obtained by stirring aflatoxin G_1 with dilute acids at room temperature. In a typical run aflatoxin G_1 (10 mg.) is magnetically stirred with 25 ml. of 5% sulphuric acid at 28° C. for 24 hr. The resulting aqueous solution is extracted six times with 20 ml. portions of chloroform; the combined extract is dried over anhydrous sodium sulphate and evaporated to 10 ml. Thin layer chromatography (TLC) of the extract using silica gel G (according to Stahl) and an eluant of 3% methanol (by vol.) in chloroform indicated the presence of two compounds. The first, fluorescing green under uv light had R_f 0·48 (corresponding to unreacted aflatoxin G_1[5]), whereas the second, fluorescing intense green had R_f 0·13. The latter was isolated from the extract by preparative TLC. An apparently unstable yellow amorphous powder, m.p. 192–194°, was obtained by crystallisation from methanol; purity of the final product is judged on the basis of appearance of a single spot on TLC using several solvent systems. The weight of the recovered material amounted to 60% of the weight of aflatoxin G_1 used.

The new compound produced intense green fluorescence in methanol and chloroform solutions. Its uv spectrum was similar to that of aflatoxin G_1[5] (maximum absorbancy in methanol at 245, 257, 263 and 362 mμ). The infrared spectrum of the substance was very similar to that of aflatoxin G_1[5] except for an additional band at 3600 cm.$^{-1}$ indicating the presence of a hydroxyl group.

Reaction of the hemiacetal with acetic anhydride in pyridine followed by pyrolysis of the resulting compound at 220° C. and at 3 mm. pressure for 10 min. afforded aflatoxin G_1 as determined by TLC.[5] This observation lends chemical evidence to the view that the OH group in the hemiacetal molecule is at position 2 of the terminal tetrahydrofuran ring, because, if it were at position 3 the preferred pyrolysis product would have been an isomer of aflatoxin G_1 with the double bond between positions 3 and 4.[6]

Co-chromatography of the new product with aflatoxin G_{2a} which was prepared by the method of Dutton and Heathcote[1,2] gave only a single spot on TLC plates, further confirming the identity of the two compounds.

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CHEMICAL RACES IN *FOENICULUM VULGARE* MILLER

DURING our studies in umbelliferous fruits a sample of fruits was supplied as *Anise* fruits (*Pimpinella anisum* L.) by the Botanist, Government Cinchona Plantation, Dodabetta, Ooty. The morphological and microscopical characters, especially the absence of epidermal hairs and presence of only six vittæ proved beyond doubt that the fruits were not *Anise* fruits. The microscopical characters resembled more to fennel though in morphological and sensory characters they even differed from fennel, in size, smell, darker colour and in their occurrence as separate mericarps and not possessing sweet taste.

The fruits and herbarium specimens were sent to Kew Gardens, England, and identified as fruits of *Fœniculum vulgare*. The volatile oil content of the fruits was much higher and upto 8%. The volatile oil did not possess the characteristic smell and taste of anethole, the chief constituent of fennel,[1] and did not show congealing. This led to the suspicion that either anethole might be present to a small

extent or it might be totally absent. By TLC studies using silica gel G according to Stahl as adsorbent and anethole and methyl chavicol as reference substance the colour tests showed the presence of methyl chavicol.[2] Separation of methyl chavicol was carried out by column chromatography and its fraction was used for infra-red spectroscopy. By I.R. of separated constituent as well as of pure sample, identity of methyl chavicol was confirmed.

Further, advantage was taken of allylic chain in methyl chavicol which was separated from anethole on TLC plates by using silica gel G according to Stahl impregnated with silver nitrate, as recommended by Nano and Martelli.[3] Only one spot corresponding to methyl chavicol was obtained and anethole was found to be absent.

Thus the fruits of *Fœniculum vulgare* collected from Ooty were found to be free of anethole and represents a *methyl chavicol race* of *Fœniculum vulgare*. Such chemical races have been found and reported in quite a few umbelliferous fruits.[4,5]

The authors' thanks are due to the Director, Kew Gardens, England, for identifying the plant and to the Botanist, Dodabetta, Ooty, for the supply of the drug and plant specimen.

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USE OF CONGLUTINATING COMPLEMENT ABSORPTION (CCA) TEST AND CCA INHIBITION TEST FOR THE DETECTION OF ANTIBODIES TO ARBOVIRUSES

ALTHOUGH various workers have reported the use of conglutinating complement absorption (CCA) test as a sero-diagnostic tool in a number of viral diseases,[1–5] there is no report on the use of this technique in sero-diagnosis of arboviruses. This communication reports firstly, the application of CCA test for detecting serum antibodies in rabbits and mice after experimental arbovirus infection and in certain convalescent humans, and secondly, the development of CCA inhibition test (CCAIT) for detecting serum inhibitory antibodies in chickens after experimental arbovirus infection.

Conglutinating Complement Absorption Test.—Serum samples were obtained from rabbits and/or mice before infection and at various intervals after infection with Japanese encephalitis (JE) virus (P 20778 strain), West Nile (WN) virus (G 22886 strain) or Kyasanur Forest disease (KFD) virus (P 9605 strain). Forty convalescent human sera taken at various intervals ranging from 11 days up to five years after onset of KFD illness from twenty-two individuals were also tested.

The hyperimmune sera against each of these viruses were raised in adult mice as per method of Clarke and Clasals.[6] All sera were inactivated at 56° C. for 30 minutes.

The viral antigens were prepared from brains of infected infant mice by the acetone-ether or sucrose-acetone extraction method of Clarke and Casals.[6] The highest dilution of antigen that reacted with any dilution of hyperimmune serum and bound a dose of complement was taken as one unit and eight units were used in tests with unknown sera.

A five-volume CCA test in tubes was employed and the tests were done by the standard procedure using normal saline as diluent, 2 to 2·5 units of horse complement and an indicator system consisting of 0·25% sheep erythrocytes and 1 : 20 dilution of bovine serum conglutinin. Primary incubation of antigen, serum (two-fold dilutions) and complement was carried out at 22° C. for 30 minutes. The indicator system in two unit volumes was then added. The complete test was incubated at 37° C. for 30 minutes and thereafter all the tubes were centrifuged at 1,500 RPM for one minute. The test was read by resuspension technique. The positive reaction was indicated by 0 to 25% conglutination and negative reaction by 50% to 100% conglutination. The appropriate controls were included in each test.

Detectable antibodies in sera from rabbits and mice were recorded as early as five to ten days after infection, which increased to reach their peak titres (1 : 32 to 1 : 128) in about three weeks' time followed by a decline and