

Dey have observed the existence of a new compound containing six molecules of ammonia.

These amino-compounds are formed by the addition of ammonium hydroxide to a solution of copper sulphate. If this addition is done gradually, a precipitate first comes down; with further addition of ammonia it goes into solution developing an intense blue colour. A study of the absorption spectra of this solution by Bhatnagar, Goyle and Prasad has shown that the main blue colour is more or less identical in nature when various concentrations of ammonia are used. This would not happen if definite compounds of different compositions are formed, that is, cupric-ammonium sulphates containing ammonia in definite different proportions do actually exist. Bhatnagar, Goyle and Prasad³ have shown that the absorption band obtained with the intensely blue-coloured solution formed by the addition of ammonia to copper sulphate is identical with that obtained with a suitably prepared colloidal solution of copper hydroxide. These observations would lead to the conclusion that the variety of the copper ammonia compounds obtained by Bhattacharya and Dey and other workers are adsorption complexes, containing different proportions of ammonia, formed by the peptising action of ammonium hydroxide or ammonium salts on copper hydroxide.

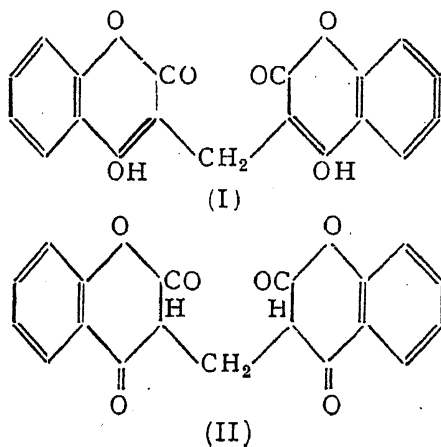
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1. *Curr. Sci.*, 1945, 14, 69. 2. *Ibid.*, 1945, 14, 201.
3. *Koll. Zeit.*, 1928, 44, 79.

ON "DICOUMARIN"—SYNTHETIC ANTI-COAGULANT

IN search for the causative agent of the hæmorrhagic disease of cattle on feeding spoiled sweet clover hay, it was noticed that 3, 3'-methylene-bis-(4-hydroxy) coumarin (I) is the substance that is acting as an anti-coagulant. It has since been synthesized from 4-hydroxy coumarin and is being suggested as an effective agent in post-operative thrombophlebitis, puerperal thrombosis, and pulmonary embolism.



It is of interest to note that this naturally occurring coumarin in sweet clover hay acting as an anti-coagulant whereas other coumarins from *E. Ayapana* are known (cf. Dymock *et al.*,² and Bose and Ray³) to act as coagulants. The above coumarin derivative (I)—also commercially known as "Dicoumarin", possesses no *in vitro* activity whereas the well-known anti-coagulant, heparin, is active both in *in vivo* and *in vitro*. Does it indicate that "Dicoumarin" is not a coumarin but a chromone of the structure (II)? Link and his collaborators⁴ have published a series of papers on the chemistry of 4-hydroxy coumarin from which this "Dicoumarin" is being produced synthetically. But the formation of its salts, its reaction with bromine, behaviour towards alcoholic ferric chloride and certain ketonic reagents, condensation with compounds containing active methylene group and many other reactions, indicate the non-existence of a hydroxy group in these 4-hydroxy coumarins. This, in other words, indicates that the compound may also exist in the form (II) when it becomes a 2-keto chromone derivative and as such may differ physiologically from the other natural coumarins as isolated from *E. Ayapana*. Details of the work are going to be published elsewhere.

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July 10, 1945.

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1. Cambell and Link, *J. Biol. Chem.*, 1941, 138, 21.
2. Dymock *et al.*, *Pharm. Indica*, 2, 245. 3. Bose and Ray, *J. Ind. Chem. Soc.*, 1936, 13, 586. 4. Link *et al.*, *Jour. Amer. Chem. Soc.*, 1943, 65, 2288 and subsequent papers.

A NOTE ON THE OCCURRENCE OF *ALCALIGENES RADIOBACTER* IN THE AERIAL ROOTS OF *PHOENIX SYLVESTRIS*

PALACIOS AND BARI, in a previous article,⁷ had reported the presence of a new organism in the nodules of *Cajanus indicus*. Since then Bergey² had also referred to it suggesting that the organism mentioned to by those workers may well be *Alcaligenes radiobacter*, despite the well-marked differences exhibited by the new species. The present workers too had an opportunity of isolating a new micro-organism from the aerial roots of *Phoenix sylvestris*, but in agreement with Bergey's opinion that no new organism should be labelled as new species (to avoid multiplication of the species), but should be, as far as possible, referred to as a variant of one of the existing species, the new organism has been named only as *Alcaligenes radiobacter*.

Phoenix sylvestris is a palm not yet adequately studied. d'Almeida and Correa¹ only recently have studied in detail the anatomy of this plant, and in agreement with the report of Kuster⁵ in connection with *Phoenix rectinata*, these workers have also observed the presence of yellowish brown contents occurring in the cortical cells of *P. sylvestris*. Richter⁸ con-

sidered these contents in *P. rectinata* as those of tannin, but the present authors observed (on microscopical examination) that the contents of *P. sylvestris* were motile. With a view to ascertain if these yellowish brown contents were living organisms, some of the aerial roots collected from different plants of *P. sylvestris* were inoculated on meat-infusion agar, Czapek agar, Congo-red media of Kellerman⁴ and of Leonard⁶ after that they had been treated by a mercuric chloride solution as recommended by Harrison and Barlow.³ The plates on incubation revealed the growth of some colonies: yellow colonies were observed on meat-infusion agar; on Kellerman's medium the colonies (whitish) were practically not absorbing the dye; on Leonard's they were clearly coloured but were not typically looking like those of *A. radiobacter*; the Czapek agar gave rise to slimy white colonies like those of *Klebsiella pneumoniae*.

The apparently different organisms isolated on these media were eventually proved to be one and the same species; the pigment appearing on meat-infusion agar was lost on Czapek for mucilage and *vice versa*. The micro-organism on routine cultural examination consistently revealed the following characteristics:—

Morphology: Rods, 0.54 to 0.75 by 1.13 to 1.41 microns, actively motile, peritrichically flagellated, non-capsulated, non-spore bearing and found in groups. Gram negative. **Infusion agar slant:** Abundant, slightly slimy, translucently greenish-yellow. **Agar colonies:** Minute, moist, raised, circular, greenish-yellow. **Czapek agar:** Abundant, slimy, white; no sign of yellow pigment. **Infusion broth:** Heavy, general turbidity, thin pellicle, mucoid deposit. **Peptone water:** Moderate, otherwise same as in the broth. **Potato:** Abundant, lemon-yellow to light red pigment. **Gelatine colonies:** Slimy, not well-pigmented; gelatine not liquefied. **Nitrates:** Reduced to nitrites. **Indol:** Not formed. **Hydrogen sulphide:** Not produced. **Sugar media:** No observable reaction; abundant growth in presence of mannite, glucose, lactose, dextrine and maltose, but not saccharose. **Starch:** Feebly hydrolysed. **Milk:** Strongly alkaline. **Pigment:** Slightly soluble in water; soluble in 95 per cent. alcohol, insoluble in chloroform, ether and carbon disulphide. **Carbohydrates agar:** Whitish colonies, very slimy instead of pigmented. Presumably mucilage prevents chromogenesis by cutting off the oxygen supply. **Carbohydrate-free agar:** Solidified peptone water proved to be a poor medium; meat-infusion agar (with sugars eliminated by *E. coli* growth) was the best for chromogenesis. **Adaptation Power:** Eight months' adaptation on infusion agar made it lose to a great extent its slime production on Czapek; the property was regained in serum-milk. **Optimum temperature:** 28° C. **Nitrogen fixation:** Slight power.

All the observations lead to the conclusion that this is a new species; but because of its occurrence in the endophytic state and of its resemblance to *A. radiobacter* (despite very many well marked differences) the authors are inclined to label this new species as a variant of *Alcaligenes radiobacter*.

SUMMARY

Presumably a new micro-organism, but labelled for convenience as a variant of *Alcaligenes radiobacter*, isolated from the aerial roots of *Phoenix sylvestris* is described at length.

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1. d'Almeida, J. F. R., and Correa, J. C., Private communication "Studies in the root-habits and anatomy of Indian Plants," *M.Sc. Thesis*, 1945. 2. Bergey, D. H., "Manual of Determinative Bacteriology," 5th Ed., 1939, Bailliere, Tindall & Cox, London. 3. Harrison, F. C., and Barlow, B., "The nodule organism of the Leguminosæ—its isolation, identification and commercial application," *Centr. Abt. Bakt. (etc.)*, II Abt., 1907, 19, 264-72, 426-41. 4. Kellerman, K. P., "The reaction of crown gall to legume inoculation." *U.S. Dept. Agr. Bur. Plant Indus. Circ.*, 1911, 76, 6. 5. Kuster, E., "Pathologische Pflanzenanatomie"—Jena, 1916. 6. Leonard, L. T., "Bacillus radiobacter in reference to commercial legume inoculants," *Jour. Bact.*, 1931, 21, 543-56. 7. Palacios, G., and Bari, A., "A new micro-organism associated with the nodule-bacteria in *Cajanus indicus*," *Proc. Ind. Acad. Sci.*, 1936, 3, No. 4, Sec. B, 362-65. 8. Richter, E., "Physiol-Anat. u. Luftwurzeln Bibliotheca," *Bot.*, 1901, 10, Heft. 54.

A PRELIMINARY NOTE ON THE ANTIBACTERIAL SUBSTANCE FROM *ASPERGILLUS FLAVUS*

WHITE (1940) first observed that *Aspergillus flavus* when grown on certain liquid medium yielded a filtrate that showed anti-bacterial activity against gram-positive and gram-negative bacteria. Glistler (1941) obtained anti-bacterial concentrate* from *Aspergillus flavus* but did not isolate it in a crystalline form. Bush and Goth cultivated *Aspergillus flavus* on the surface of the liquid medium and obtained an anti-bacterial substance. This substance which is soluble in ether and water was called Flavacin. It was extracted by the authors with isopropyl ether in an atmosphere of carbon dioxide. The crude product, toxic to mice, was found to be innocuous after purification. Waksman and Bougie (1943) used six strains of *Aspergillus flavus* and five of *Aspergillus flavus orizae*, the latter yielding little or no anti-biotic substance. According to them two factors were responsible for the anti-biotic activity, namely, aspergillic acid, which is active both against gram-positive and gram-negative bacteria and flavacin mostly active against gram-positive bacteria and, therefore, very similar to penicillin. The strain of *Aspergillus flavus* under submerged condition of growth, produced enough flavacin to be compared favourably with penicillin produced by the best strain of *Penicillium notatum* grown under similar conditions. McKee and Mac-Phillany (1943) found from *A. flavus* antibacterial substance unlike aspergillic acid and closely resembling penicillin in its biological and chemical nature. Menzel *et al.* (1943) gave