way followed depended on the enzyme acting and the *p*H prevailing.

(I), or its enzymatic breakdown products, slowly induced β-galactosidase formation in *E. coli* (*K* 12). Details of enzyme action on (I) will be published elsewhere.

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Pyrethrin and Gallotannin in the Flowers of Peony

From a light petroleum extract of dried petals of peony (*Peonia albiflora* Pall.) an oily substance (74·08 per cent C, 9·94 per cent H) was isolated by means of nitromethane extraction. It comprises 0·13 per cent of the dry weight of petals and 18 per cent of light petroleum extracts and shows in the ultraviolet spectral region maxima at 227 and 280 mμ —characteristic of pyrethrins[1]. Alkaline hydrolysis of this substance gives: chrysanthemic acid (oil, volatile in steam) and chrysanthemum dicarboxylic acid (m.p. 205–206°) in the ratio of 1 : 1. Paper chromatography in light petroleum saturated with methanol[2] extract of the compound investigated and of pyrethrins, isolated from *Chrysanthemum cinerariefolium*, as well as of the products of their hydrolysis (acids and rhetrolones), afforded a proof of the identity of both substances.

In the methanol extract from defatted petals, gallic acid derivatives were found in great quantities. From this extract a substance, comprising 10 per cent of the dry weight of petals, was separated, being soluble in ethyl acetate and acetone, but insoluble in dry ethyl ether. It possesses tannin-like properties and after hydrolysis gives gallic acid as the only phenolic compound and glucose as the only carbohydrate, detected by paper chromatography. As the result of methanolysis[3] the methyl gallate was identified. Infra-red absorption of the compound, methylated with diazomethane, indicated the absence of free hydroxy groups, which indicated a structure of galloylated pentagalloylglucose for the compound investigated. The total amount of acid liberated on hydrolysis of the methylated compound was determined by titration, and the quantity of di-*O*-methylgallic acid found by measurement of the colour developed with Folin's reagent. The ratio of the two methylated gallic acids, 5 : 1, confirmed that the compound investigated is *m*-mono-galloyl-(pentagalloylglucose).

These results are interesting because it is the first observation of the occurrence of pyrethrins and gallotannins in a plant belonging to Ranunculaceae. The first of these compounds was hitherto found only in the *Chrysanthemum* genus (Compositae); the second one has not previously been found in flowers.

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Cross-dependence between Neamine and other Basic Antibiotics

The emergence of streptomycin-dependent as well as 'persisters'[1,2] and resistant bacteria in an environment containing streptomycin is well known. Studies on the mode of action of streptomycin-dependence have been presented in a number of recent publications[3–6]. This communication adds to the present knowledge of antibiotic dependence and the relationship between structure and biological activity.

A strain of *Staphylococcus aureus FDA* 209*P* resistant to 1 mg neamine per ml. was obtained after approximately 20 serial sub-cultures into nutrient broth with increasing concentrations of neamine per transfer. After 20 additional transfers in nutrient broth containing 1 mg neamine per ml., the culture was neamine-dependent. Good growth occurred after 24 h at 32° C with concentrations of neamine between 20 and 1,000 μg/ml., light growth with 1–20 μg/ml. and no visible growth without neamine.

Tests were conducted to determine if the requirement for neamine could be fulfilled by other antibiotics. Each antibiotic was added to nutrient broth to concentrations of 1, 2, 5, 10, 20, 50, 100, 200 and 500 μg/ml. One-tenth ml. of a 1 : 100 dilution of a 24-h culture of the requiring strain was added to each tube containing 10 ml. and all tubes were incubated at 32° C. Growth occurred with streptomycin, paromomycin, zygomycin, neomycin C and neamine (Table 1). No growth occurred with neomycin B, kanamycin, erythromycin, spectinomycin and vancomycin. A response of growth indicated that the organism utilized the antibiotic as a substitute for its neamine requirement. Growth could not be interpreted as antibiotic resistance since growth did not occur in control tubes without antibiotic. Three control tubes without antibiotic were included in each broth experiment.

Tests for requirement were also conducted on plates containing nutrient agar seeded with 2 ml. of the dependent culture per 100 ml. of medium. Paper disks were impregnated with antibiotics and placed on the agar surface and the plates were incubated at 32° C for 24 h. Zones of growth around the disks were evidence of growth support. The agar tests confirmed the broth results. Fig. 1 illustrates the growth response of neamine and two other antibiotics.

Table 1 gives structures of the antibiotics tested and their effect on the growth of the neamine-requiring strain of *S. aureus*. Note that the common factors among the antibiotics that can substitute for neamine are: (1) aminohexoses; (2) nitrogen groups in the 2-carbon position on the hexoses. Basic antibiotics with no amino-sugars, such as spectinomycin (reported as actinospectacin in the literature) and vancomycin, and basic antibiotics with aminohexoses but with the nitrogen in the 3-carbon position, such as kanamycin and erythromycin, did not substitute.

Neomycin B did not substitute for neamine even though it contains 2,6-diamino-D-glucose, the same as neamine. However, the other amino-sugar is probably an active antibacterial site which may prevent the growth of the organism.

A streptomycin-dependent strain of *S. aureus* (derived from *P.S.* 53), which was obtained through the courtesy of Dr. Ruth Z. Korman at the School of Veterinary Medicine, Cornell University, was tested for cross-dependence. This strain acted