8.5; analysis: C, 54.56; H, 5.34; N, 5.77; Cl, 7.16; O, 21.17 (by diff.); mol. wt., 508 (3).

Aureomycin forms a hydrochloride with these properties: decomposes above 210° C;  $[\alpha]_{D^{23^{\circ}}}$ , -240.0 (water); approx. solubility in water, 14 mg/ml at 25° C; pH of aqueous solution, 2.8-2.9; analysis: C, 51.84; H, 5.24; N, 5.46; total Cl, 13.27; ionic Cl, 6.69; O, 24.19 (by diff.). The rhomboid crystals have a refractive index of  $1.700 \pm 0.005$ . The acute angle is  $80 \pm 5^{\circ}$ .<sup>2</sup>

In 0.1 N hydrochloric acid, aureomycin shows absorption maxima at 230, 262.5, and 367.5 mµ. In 0.1 N sodium hydroxide the maxima are at 255, 285, and 345 mµ.

#### References

- 1. BRALEY, A. E., and SANDERS, M. J. A. M. A., 1948, 138, 426.
- 2. BRYER, M. S., SCHOENBACH, E. B., CHANDLER, C. A., BLISS, E. A., and Long, P. H. J. A. M. A., 1948, 138, 117.
- CLARK, E. P. Ind. eng. Chem. (Anal. ed.), 1941, 13, 820.
   DUGGAR, B. M. Ann. N. Y. Acad. Sci., 1948, 51, 177.
- 5. PAINE, T. F., JR., COLLINS, H. S., and FINLAND, M.  $J_{\cdot}$
- Bact., 1948, 56, 489. 6. WRIGHT, L. T., SANDERS, M., LOGAN, M. A., PRIGOT, A.,
- and HILL, L. M. J. A. M. A., 1948, 138, 408.

# 2-Acetylamino-9-C14-Fluorene1

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Since it was demonstrated by Wilson, DeEds and Cox (4) that 2-acetylaminofluorene causes a wide variety of cancer in rats, this compound has become of increasing importance in experimental cancer research (1-4). Present chemical methods are successful in accounting for only about one-third of the substance administered (3). With the hope of completely elucidating the mode of action of 2-acetylaminofluorene we have synthesized it with radioactive carbon-14 in the 9-position in the molecule.



Measurements were made with a Geiger tube having a window 4-5 mg/cm<sup>2</sup> and at a distance of  $1\frac{1}{3}$ ".

The following equations give the yields and number of counts per minute at each step for a 22 mg/cm<sup>2</sup> sample from 2 mc of BaC<sup>14</sup>O<sub>8</sub> and 11.5 gm of 2-iodobiphenyl.

2-lodobiphenyl 
$$\xrightarrow{87\%}$$
 2-biphenylmagnesium iodide  $\xrightarrow{60\%}_{C^{14}O_2}$   
20,897 cpm 88% 24,872 cpm 70%  
2-C<sup>14</sup>-biphenylcarboxylic acid  $\rightarrow$  9-C<sup>14</sup>-fluorenone  $\rightarrow$   
23,630 cpm 79% 20,969 cpm 90%  
9-C<sup>14</sup>-fluorene  $\rightarrow$  2-nitro-9-C<sup>14</sup>-fluorene  $\rightarrow$   
24,832 cpm 84% 21,341 cpm  
2-amino-9-C<sup>14</sup>-fluorene  $\rightarrow$  2-acetylamino-9-C<sup>14</sup>-fluorene

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<sup>2</sup> We wish to thank E. F. Williams, of the Stamford Laboratories, American Cyanamid Company, for the crystal analysis, and L. M. Brancone and staff for the microanalyses.

The metabolism of the radioactive compound is being studied by H. P. Morris, of the National Cancer Institute, and the results will be reported in a subsequent publication.

#### References

- 1. ARMSTRONG, E. C., and BONNER, G. M. J. Path. Bact., 1944, 56, 507-512; Brit. J. exp. Path., 1946, 27, 97.
- 2. BIELSCHOWSKY, F. Proc. biochem. Soc., 1943, 37, 15; Brit. J. exp. Path., 1944, 25, 1, 90; Biochem. J., 1945, 39, 287.
- 3. WESTFALL, B. B. J. Nat. cancer Inst., 1945, 6, 23.
- WILSON, R. H., DEEDS, F., and Cox, A. J., JR. Cancer 4. Res., 1941, 1, 595; 1947, 7, 647.

# **Experiments in Crossing** Aedes (Stegomyia) aegypti Linnaeus and Aedes (Stegomyia) albopictus Skuse<sup>1</sup>

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In 1937, Toumanoff (4) reported a series of crosses made between the two species of mosquitoes, Aedes aegypti and Aedes albopictus. In two trials he had two successful crosses of albopictus females and aegypti males. The offspring resembled albopictus. The F2,  $F_3$ , and  $F_4$  generations from these crosses also resembled albopictus. In four trials with aegypti females and albopictus males he had only one success, the offspring and  $F_{a}$  generation resembling aegypti. Later (5) he reported five more crosses of albopictus females and aegypti males, two of which were successful, the  $F_1$  again resembling the female parent. Hoang-Tich-Try (2) also reported four attempts at crossing albopictus females and aegypti males; two of the trials were successful, the  $F_1$  resembling albopictus. He did not succeed in getting an F<sub>2</sub> generation. Edwards (1) comments on this work, suggesting the desirability of more detailed morphological studies of the crosses.

In 1944, Johannes Bauer tried to confirm this work, using colonies of A. aegypti and A. albopictus which had been maintained in the New York Laboratories of the International Health Division of The Rockefeller Foundation for several years. The origin of these colonies is not known. It was considered possible that, in the course of innumerable transfers of eggs and larvae, some mixing of the two species might well have occurred from time to time. Evidence of this was obtained, for upon examination of a large number of specimens from the cage of either colony, an occasional member of the other species would be found. Consequently, it can be suggested that neither of the lines was necessarily "pure." After preliminary trials, Dr. Bauer turned the project over to the

<sup>1</sup> Work conducted under the auspices of U.S. Naval Medical Research Unit No. 2 in the Laboratories of the International Health Division of The Rockefeller Foundation, New York. The Navy Department does not necessarily endorse the views set forth in this paper.

<sup>2</sup> The authors were formerly with NAMRU No. 2.

Tests were carried out in an insectary with an average temperature of 75° F. The mosquitoes were placed in cages with two layers of screening  $\frac{1}{2}''$  apart, with the front opening covered by heavy muslin cloth. Sugar water was kept in the cages, and every third day a chick was introduced into each cage to provide a blood meal. Eggs were placed in containers in screened cages for hatching and the rearing of larvae. Each pupa was removed and put into a separate test tube, and each adult transferred to a separate dry tube as it emerged. The

#### TABLE 1

CROSSING ATTEMPTS WITH A. albopictus and A. aegypti Mosquitoes

Caged	Progeny	Observations
Trial A, female A. albopictus and male A. aegypti		
Group 1 13 ♀ × 7 ♂	None	A few eggs, none hatched
Group 2 59 ♀ × 37 ♂	None	Some eg <b>gs,</b> none hatched
Group 3 170 ♀ × 112 ♂	None	A few eggs, none hatched
Group 4 59 ♀×123 ♂	None	No eggs
Trial B, female A. aegypti and male A. albopictus		
Group 1. $8 \ 2 \times 10 \ \sigma$ F <sub>2</sub> generation	54 ♀ and 104 ♂	
$44 \mathbf{F}_1 \mathbf{Q} \times 83 \mathbf{F}_1 \mathbf{d}$	11 Q and 31 $\sigma$	
Group 2 52 ♀ × 28 ♂	None ·	Numerous eggs, none hatched
Group 3 105 ♀ × 42 ♂	$2$ $_{\mathcal{C}}$	Experiment interrupted
Group 4 61 ♀ × 162 ♂	14 Q and $45 \sigma$	

males and females to be introduced into cages were routinely checked under a low-power microscope by two observers.

A binocular dissecting microscope was used to check distinguishing markings of the offspring of the crosses. The pattern on the mesonotum, the sides of the thorax, the legs, the abdomen, the head, and the palpi was carefully examined. A. aegypti has a characteristic lyre pattern and A. albopictus a broad central band on the mesonotum. These are the principal distinguishing marks. The patterns of silver scales on the occiput and on the sternopleura and mesepimeron are also different. Combscale characters of the larvae were checked. Male terminalia were dissected out and examined. Particular attention was paid to the morphology of the ninth tergite, which is markedly different in the two species.

Experiments were started on August 5, 1944, and terminated on October 17, 1944. The experiments are summarized in Table 1. The *albopictus* females of trial A did not feed readily on blood, while the *A. aegypti* females in trial B took blood readily. Sperm was found in the spermatheca of only one of 24 albopictus females (trial A, group 3) dissected, although copulation had been observed. In group 2 of trial B, copulation was observed and of three aegypti females examined on October 17, one had sperm in the spermatheca, although no eggs were hatched. All of several aegypti females from group 4 of trial B, examined on October 12, had sperm in their spermathecae.

All of the offspring, including the  $F_2$  generation of group 1, trial B, resembled *A. aegypti* in every detail. The first adult  $F_2$  were observed on October 17, but the experiment had to be terminated at that time.

It is noteworthy that in the experiments of Toumanoff and of Hoang-Tich-Try, as well as in our own tests, offspring of the crosses have resembled the female parent. In our work reported above, this resemblance held true down to the finest morphological details of larvae and adult mosquitoes which it was possible for us to check.

It is difficult to explain these results on a genetic basis. One possibility is that fertilization by the male of the other species was not a true fertilization, but served to stimulate parthenogenetic development of the ovum. Be this as it may, both male and female offspring were obtained.

It is interesting that Summers Connal (3) working on variations observed in A. *aegypti* in Lagos, Nigeria, has noted an extensive range of color variations (the lyre pattern remaining constant). The possibility that A. *aegypti* will cross with closely related species in nature is suggested.

#### References

- EDWARDS, F. W. Mosquitoes of the Ethiopian region. (Pt. 3.) (Natural History), British Museum, London: 1941. P. 130.
- 2. HOANG-TICH-TRY. Bull. Soc. path. exot., 1939, 32, 511.
- SUMMERS CONNAL, S. L. M. Bull. entomolog. Res., 1927– 28, 18, 5.
- TOUMANOFF, C. Bull. Soc. méd. chir. de l'Indochine, 1937, 51, 964.
- 5. TOUMANOFF, C. Bull. Soc. path. exot., 1939, 32, 505.

# Effectiveness of Vitamin P Compounds in Counteracting Anticoagulant Action of Dicoumarol

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Campbell (4) and Overman, et al. (8) demonstrated in the rabbit that 2-methyl-1, 4-naphthoquinone counteracted the action of dicoumarol. Overman, et al. (8) also reported the ability of ascorbic acid to reduce the hypoprothrombinemic response to dicoumarol. Later, this Wisconsin group (1) found that dicoumarol increases the excretion of ascorbic acid in the rat.