Technical Papers

Biosynthesis of Penicillin. I. Role of Phenylacetic Acid

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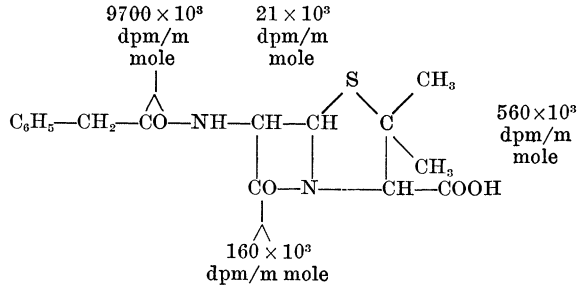
In view of the long period that penicillin has been under investigation it is somewhat surprising that comparatively little is known about the biosynthesis of this antibiotic. The only tracer studies that have come to our attention are those utilizing deuterophenylacetyl-N^{15}-valine (*1*), phenylacetic acid-1-C^{13} (*2*), and S^{35} (*3*). The apparently clear-cut results obtained in the incorporation of the benzyl residue from phenylacetic acid into benzylpenicillin have perhaps discouraged further investigations with this precursor.

Behrens *et al.* (*1*) found that 92.5% of the benzylpenicillin obtained was derived from the deuterium-labeled phenylacetic acid. Craig *et al.* (*2*) similarly found excellent incorporation of phenylacetic acid-1-C^{13} into benzylpenicillin, although quantitative data are not given.

We have studied the incorporation of phenylacetic acid-1-C^{14} into benzylpenicillin by *Penicillium chrysogenum* Wis 49-133 and have found that the specific activity of the phenylacetic acid isolated by chemical degradation of the penicillin (*4*) was about 82% of the activity of the precursor phenylacetic acid. Decarboxylation (*5*) of the phenylacetic acid isolated by hydrolysis of the penicillin showed all of the radioactivity to be in the carboxyl group. We have also investigated other major fragments of the penicillin molecule by chemical degradation (*4*) and the results are summarized below:



The starting phenylacetic acid had an activity of $11{,}500 \times 10^3$ disintegrations/min/m mole. The specific activity of the penicillin was approximately $12{,}000 \times 10^3$ disintegrations/min/m mole. All activity values have a standard deviation of 5% or less, except for the figure given for the glycine moiety (21×10^3) which has an S.D. of about 10%.

A rough correspondence has been observed between the average specific activity of the respiratory CO_2 from the fungus and that of the CO_2 from the β-lactam carbonyl, and this similarity points to the possibility of CO_2 fixation playing a role in penicillin biosynthesis (*6*). These results are apparently not in agreement with those of Godzesky, Martin, and Stone (*7*), who report no significant fixation of $C^{14}O_2$ from bicarbonate into the penicillin molecule by *P. chrysogenum* (Q-176). Work on determination of the individual specific activities of all the carbons in this biosynthetic penicillin is in progress. Fermentation details will be described separately.

References

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The Reaction Occurring in CO_2–H_2O Mixtures in a High-Frequency Electric Arc[1]

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This study was initiated in the hope of isolating 1, and possibly 2, carbon-atom compounds formed by the reduction of carbon dioxide by water vapor in a high-frequency arc. It forms a part of a series of studies of the chemical effects of high-frequency discharges on gaseous systems. The action of radiation on these 2 gases is of special interest in relation to the basic photosynthetic process, and also carries implications with respect to the origin of living matter on earth. A typical flow system was employed, with an army aircraft 150-w transmitter supplying the necessary power to the discharge tube.

Exhaustive examination has failed, however, to definitely establish the presence of any interesting reduction products in a reproducible way. Most of the parameters affecting the process were investigated, including current, pressure, flow rate, and H_2O/CO_2

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ratio, but without definitive results. The ranges of these variables are given in Table 1.

TABLE 1

Variable	Range
Flow rate	5–35 cc/sec (STP)
Pressure	50–250 mm Hg
Current	75–200 ma
Composition	3–25% H_2O by volume
Voltage	500–600 v
Gap distance	5 mm
Frequency	3 mc
Electrodes	Copper and Inconel

The following methods of analysis were employed:

1. Infrared spectra of the product gases were examined in the range of wavelengths of 2.5–15 μ with a Perkin-Elmer model 12C automatic-recording spectrophotometer. The only compounds having absorption bands in the above region which could be definitely identified were CO_2, H_2O, and CO.

2. To meet with the sensitivity of the chemical tests employed in detecting the products of the irradiation, the duration of runs was increased from 4–90 min. The exit gases from the discharge tube were thoroughly scrubbed in liquid-water traps. Since all of the likely products (formaldehyde, formic acid, carbon suboxide, glyoxal, etc.) are soluble in water, this method should collect nearly all of the products of interest. In general, no positive tests were obtained with, e.g., dilute permanganate, modified Schiff's reagent (for formaldehyde [*1*]) and dimethyldihydroresorcinol (*2*), a standard reagent for formaldehyde. Tests for formic acid and methyl alcohol (*3*) also gave negative results. Occasionally the ice from the dry ice-acetone trap or washings from the walls of the discharge tube decolorized permanganate solution but still gave no specific compound tests. In an effort to recover monomers from possible polymeric products present, several samples were made basic and digested to depolymerize any substances such as paraformaldehyde. Again, no positive results were obtained.

3. The product gases were passed through 1% sodium bisulfite solution according to the procedure of Goldman and Yagoda (*4*) for estimation of traces of formaldehyde in air. The excess bisulfite is titrated with 0.1 *N* iodine solution in acid medium. This solution is made basic with Na_2CO_3–acetic acid buffer and the formaldehyde liberated from the bisulfite complex is titrated with 0.01 *N* iodine solution. Sensitivity of the order of 7 parts (by wt) per million is claimed in this method. No formaldehyde could be detected, however.

In summary, it appears that under the conditions employed, no interesting reduction products of the carbon dioxide–water reaction are formed, at least in quantities greater than 10 ppm. Traces of the usual matter found in discharges tubes—solid polymers of unknown composition—may be formed, however.

The reason for the nonappearance of reduction products in chemically significant quantities is not clear. The decomposition of CO_2 to CO took place to the extent of about 9%, and it is known (*5*) that CO and hydrogen atoms (produced from H_2O) react in discharge tubes to give formaldehyde. Either there was an insufficient supply of hydrogen atoms, or any product formed was decomposed thermally in the region of the arc plasma itself or at the electrodes.

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Susceptibility of *Rattus hawaiiensis* Stone to Warfarin

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Previous cage tests (*1*, *2*) have shown that warfarin-treated rolled oats were readily accepted by the five rodent species, *Rattus norvegicus* (Erxleben), *Rattus alexandrinus* (Geoffrey), *Rattus rattus* Linn., *Rattus hawaiiensis* Stone, and *Mus musculus* Linn. present in the Territory of Hawaii and that all these species died from the effects of the poison.

Field tests reported by Doty (*1*) showed that warfarin-treated oats gave good control of rats damaging sugar cane, and he suggested that for satisfactory results the warfarin bait should be exposed for at least 17 days or until the consumption of bait reached zero.

Gross, Baker, and Bonnet (*3*) in a large scale field test in an endemic plague region in Hawaii, have shown that all species of rodents were adequately controlled with the same bait formula except the local endemic species, *Rattus hawaiiensis*. The importance of this species in the epidemiology of rodent plague in the Territory of Hawaii (*4*) indicated the desirability of investigating further the susceptibility of this species to warfarin-poisoned rolled oats.

A series of cage feeding tests was instituted after the procedures previously used. Specimens of *Rattus norvegicus* and *Rattus hawaiiensis* were captured alive near Honolulu and confined in laboratory cages. A record was kept of the sex and weight. The animals were provided with pieces of coconut, plain rolled oats, and unlimited water. After a short period of adaptation to the cages each rat was presented with a weighed quantity of warfarin-treated oats.[1] The poisoned oats were removed and replaced with unpoisoned rolled oats every other day. The amount of food or bait eaten was determined by daily weighing of the food dish and its contents. The warfarin bait was regularly accepted and there was no evidence of consistent bait refusal or avoidance during the test. This

[1] A commercial product prepared according to the formula of Doty (*1*) and containing by weight 0.025% warfarin, 11.0% white mineral oil, 0.25% *p*-nitrophenol (a mold deterrent) and 88.73% rolled oats. This is identical with the warfarin bait used in previous tests (*1–3*).