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Radicinin: A Metabolite from *Stemphylium radicinum.*
I. Chemistry and Action

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**INTRODUCTION**

In previous papers from this laboratory, reports have been made regarding the correlation of the structure and biological action of certain mold pigments (1-3). Continuing these investigations, it appeared interesting to examine metabolites from genera which have not been extensively studied. For this purpose the mold *Stemphylium radicinum* (Sterad) was chosen. The organism was originally described under the name *Alternaria radicina* (4). Its occurrence and pathological effects have been reported by Grogan and Snyder (5).

This organism was observed to secrete a yellow pigment which crystallized in the growth medium after the cultures had been standing in the incubator for about 3 weeks. Following a preliminary report (6), a detailed description of the isolation and evidence regarding the structure of this metabolite, for which we proposed the name *radicinin,* will now be given.

**STRUCTURE OF RADICININ**

Microanalyses and molecular-weight determinations pointed to the formula $C_{12}H_{12}O_{5}$ for radicinin. It was optically active and contained two terminal methyl groups, but no methoxyl groups. Radicinin was insoluble in sodium bicarbonate or sodium carbonate solutions but dis-
solved in dilute sodium hydroxide to give a pink color. It was thus a weak acid.

A hydroxyl group was indicated by the formation of a monoacetate.\(^2\)

The presence of a carbonyl group was detected by the slow formation of a mono-2,4-dinitrophenylhydrazone. Tests for the aldehyde group were negative as were also those for the methyl ketone group. However, this compound reduced Fehling’s solution on boiling, and Tollens’ solution on warming.

Bromine and hydrogen chloride added to radicinin slowly. The halogen atoms in these derivatives were inert toward alcoholic silver nitrate, and both compounds formed monoacetates. When the bromine adduct was treated with sodium iodide in acetone, free iodine was liberated. This confirmed the fact that addition had occurred. If an excess of bromine was used, substitution occurred as shown by evolution of hydrogen bromide.

Radicinin absorbed 3.4 moles of hydrogen on prolonged catalytic hydrogenation in glacial acetic acid plus methanol (1:1) as solvent with Pd on charcoal as catalyst. Under the same conditions, radicinin hydrogen chloride absorbed 2.3 moles of hydrogen.\(^3\)

Pyrolysis of radicinin gave rise to 1 mole of CO\(_2\) per mole of added pigment. This established the presence of a potential carboxyl group.

When radicinin was refluxed in dilute alkali under an atmosphere of nitrogen (99.7% pure), acetaldehyde could be trapped in the effluent gases as its 2,4-dinitrophenylhydrazone. Quantitative experiments showed that 0.4 mole of acetaldehyde was obtained for each mole of starting material. After cooling and acidification, 0.4 mole of CO\(_2\) was recovered. In addition 0.3 equiv. of volatile acid could be obtained from the cooled reaction mixture. All attempts to isolate larger fragments of the molecule in this degradation procedure were of no avail. A tan-colored resin was obtained, which could not be purified.

If the nitrogen in the above experiment was scrupulously freed from oxygen by passage over heated copper gauze, only a trace of acetalde-\(^2\) 

Owing to the high acetyl values obtained on this derivative, radicinin was at first thought to be a diacetate (6). These high results have been shown to be caused by the decomposition of radicinin itself to yield volatile acid under the conditions of the hydrolysis.

This excludes the possible presence of a benzene ring. The structure suggested in a previous communication (6) thus required modification. The fractional moles of hydrogen added may be due to hydrogenolysis of C—O bonds.
hyde was trapped; hence an oxidation was involved in the production of acetaldehyde.

Radicinin consumed large quantities of oxidizing agents such as potassium permanganate, ozone, and chromic acid, and only small fragments of the molecule remained. However, the dibromide of radicinin gave $\alpha,\beta$-dibromobutyric acid on oxidation with potassium permanganate. This acid was the stereoisomer which is obtained by the bromination of trans-crotonic acid (7). The presence of the group $\text{CH}_3\text{H}^\text{C}=\text{C}$ is thus established.

A methyl ether of radicinin was obtained by treatment with dimethyl sulfate and potassium carbonate in acetone.

Alkali fusion of radicinin yielded a trace of degradation products which were not identified (8). These gave positive Gibbs' tests (9) and thus appeared to be phenolic.

On the basis of the chemical evidence, we could now account for the presence of a free hydroxyl group, three carbon-to-carbon double bonds, a carbonyl group, a potential carboxyl group, and two terminal methyl groups. The ring system found in radicinin remained to be established.

The infrared absorption spectra of radicinin (Fig. 1) and of its derivatives confirmed the conclusions arrived at from the chemical data. Radicinin has an absorption band at 2.90 $\mu$ indicating the presence of a hydrogen-bonded hydroxyl group. There is a band at 6.02 $\mu$ which points to a conjugated carbonyl group and one at 5.66 $\mu$ which suggests the existence of a five-membered lactone ring. The bands at 6.21 and 6.55 $\mu$ showed the presence of conjugated carbon-to-carbon double bonds (10). When the spectrum of radicinin was measured in chloroform solution, there was no shift in the position of the above bands. Hydrogen bonding is, therefore, intramolecular.

When radicinin was acetylated, the band at 2.90 $\mu$ disappeared, while a new one appeared at 5.75 $\mu$, as is to be expected for an acetate (10). Both the bromine and hydrogen chloride adducts showed the same strong carbonyl bands found in radicinin at 5.66 and 6.02 $\mu$.

In the ultraviolet region, the spectrum of radicinin had an intense absorption maximum at 343 $\text{m}\mu$ ($\log \varepsilon, 4.27$) and less intense ones at 270 $\text{m}\mu$.
Fig. 1. Infrared absorption spectrum of radicinin.

(log $\varepsilon$, 3.79) and 280 m$\mu$ (log $\varepsilon$, 3.62) (Fig. 2). There is an additional peak at 220.5 m$\mu$ (log $\varepsilon$, 4.22). This indicated extensive conjugation in the radicinin molecule.

The ultraviolet absorption spectrum of the acetate was almost identical with that of radicinin as shown in Fig. 2. Acetylation, therefore, did not change the chromophoric group responsible for the ultraviolet absorption spectrum of radicinin. This suggested that the hydroxyl group was not conjugated with the chromophore.

Since there were two conjugated carbon-to-carbon double bonds in the molecule, and an atom of oxygen had not yet been accounted for, the furan and pyran rings were considered to explain the properties of radicinin. However, a hydroxyfuran or pyrone formulation would not be consistent with the fact that radicinin does not give a ferric chloride test, and that the ultraviolet absorption spectra of radicinin and its acetate are nearly identical. Patulin and its acetate have nearly identical ultraviolet absorption spectra (11) and hence resemble radicinin in this respect.

By a process of elimination, the only structure which seemed to accommodate simultaneously all the evidence obtained so far for the structure of radicinin was the following:

```
\begin{align*}
\text{H}_2\text{C} & \quad \text{H} \\
\text{C} & \quad \text{C} \\
\text{H} & \quad \text{C} \\
\text{C} & \quad \text{C} \\
\text{H} & \quad \text{O} \\
\text{H}-\text{O} & \quad \text{CH}_3 \\
\text{O} & \quad \text{3H}
\end{align*}
```

The lactone ring is stable in acid while the hemiacetal is not, and the
hemiacetal is stable in alkaline medium while the lactone ring is not. Position 9 is thus a stable asymmetric center and this enables the isolation of optically active radicinin (11).

In addition, the infrared data are consistent with such a formulation. An $\alpha,\beta$-unsaturated five-membered lactone ring explains the band at 5.66 $\mu$ (12), and an $\alpha,\beta,\alpha',\beta'$-unsaturated carbonyl group is consistent with the band at 6.02 $\mu$. The conjugated double bonds explain the origin of the bands at 6.21 and 6.55 $\mu$. Finally, the position of the hydroxyl group makes possible hydrogen bonding as indicated by the band at 2.90 $\mu$.

While there were no easily available model compounds with which the ultraviolet absorption spectrum of radicinin could be compared, there were some similarities in the chromophoric group between terramycin\(^4\) with maxima at 357 and 267 $\mu\mu$ (13) and radicinin with maxima at 343 and 270 $\mu\mu$.

The molecular dimensions, as determined by x-ray diffraction measure-

\(^4\) The benzene ring is assumed to have approximately the same effect on the ultraviolet absorption spectrum as one double bond (13a).
ments, were also consistent with the above formula. The following unit cell dimensions, in angstrom units, were determined for radicinin and its hydrogen chloride and bromine adducts:

Radicinin: $a$, 6.50; $b$, 8.07; $c$, 21.56;
Radicinin.HCl: $a$, 5.06; $b$, 9.74; $c$, 25.15;
Radicinin.Br$_2$: $a$, 5.46; $b$, 10.70; $c$, 25.42.

The position of the remaining methyl group could not be determined on the basis of the evidence now available. It may be located either at positions 3, 5, 7, or 9.

The additional observation was made that, while the ultraviolet absorption spectrum of radicinin was not affected by the addition of acid, it underwent an irreversible transformation when the spectrum was measured in alkali (Fig. 3). There first occurred a bathochromic shift,

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These values were measured for us through the courtesy of Dr. Ray Pepinsky of the Pennsylvania State College.
which varied with the nature of the solvent. With methanol the first peak in radicinin was shifted from 343 to 365 m\( \mu \). This peak decreased with time, and new peaks appeared at 315 and 255 m\( \mu \). The peak at 315 m\( \mu \) appears to be due to the formation of a transient intermediate, while that at 255 m\( \mu \) suggests that a cyclic \( \beta \)-diketone was being formed (14). The end product of this reaction was not isolated, as only gums were obtained, and no further conclusions could be drawn as to its identity. However, this evidence would seem to favor placing the remaining methyl group either at position 7 or 9.

Radicinin hydrogen chloride and radicinin dibromide\(^8\) showed similar absorption spectra (Fig. 4). The first absorption maximum was shifted back from 343 m\( \mu \) (log \( \varepsilon \), 4.27) in radicinin, to 310 m\( \mu \) (log \( \varepsilon \), 4.07) and 318 m\( \mu \) (log \( \varepsilon \), 4.05), respectively. This hypsochromic shift coupled with a hypochromic effect is consistent with the shortening of the conjugated path of the chromophore which was to be expected when the double bond

\(^8\) When dibromoradicinin was treated with sodium iodide in acetone, a product which contained no iodine was obtained. Its ultraviolet absorption spectrum was identical with that of radicinin.
of the crotonyl group had been saturated. The ultraviolet absorption spectrum of dihydroradicinin ($\lambda_{\text{max}}$ 310 m$\mu$, log $\epsilon$, 4.04) was very similar to that of radicinin hydrogen chloride. In addition, it showed a bathochromic shift and irreversible transformation similar to radicinin, when the spectrum was measured in alkali.

Radicinin methyl ether had an absorption maximum at 334 m$\mu$ (log $\epsilon$, 4.15) while the dinitrophenylhydrazone showed a maximum at 416 m$\mu$ (log $\epsilon$, 4.38). This indicated that the ketone group was part of an extended conjugated system (15).

In addition, it was observed that no ketonic derivatives were obtained with phenylhydrazine or hydroxylamine. This suggested that the hemiacetal linkage was split prior to the formation of the 2,4-dinitrophenylhydrazone, and that the carbonyl group which reacted was that potentially present at position 7. This is supported by the low value of the optical rotation of this derivative.

Further studies on the catalytic hydrogenation of radicinin showed that when Pd on charcoal was the catalyst, the product obtained had an absorption maximum at 263 m$\mu$, and this maximum was shifted to 290 m$\mu$ in the presence of 0.1 $N$ alkali. In addition, this product gave an amber color with ferric chloride. It, therefore, appears to be a $\beta$-dicarbonyl compound.

When the hydrogenation was carried out in the presence of platinum dioxide, the oil obtained had an absorption maximum at 283 m$\mu$, and this peak was not shifted in the presence of alkali. This product did not give a color reaction with ferric chloride.

All of this additional evidence serves to corroborate the structure which is presented for radicinin.

**Studies on the Action of Radicinin**

It was previously observed in this laboratory that rubrofusarin and related xanthones could affect the rate of dehydrogenation of isopropyl alcohol to acetone by *Fusarium lini* Bolley (FIB) (1). Xanthone itself was observed to cause a slight increase in the rate of dehydrogenation, while the natural pigment retarded the progress of the dehydrogenation. Weiss and Nord (2) made a similar observation with the pigment solani-mone, a naphthoquinone derived from *Fusarium solani* D$_2$ purple. It decreased the rate of dehydrogenation without inhibiting the growth of the test organism. The effect of radicinin on this system was therefore studied. In contrast to the results obtained with the above-mentioned
pigments, radicinin caused an increase in the rate of dehydrogenation of isopropyl alcohol by FlB. The rate of acetone formation was 1.9 mg./day in the controls, while that in the presence of radicinin was 3.5 mg./day. There was also a slight stimulation in the growth of the test organism as shown by a 30% increase in the mycelial weights in the presence of radicinin over the controls at the end of the experiment.

It was observed that when solanione was added to the growing nonpigment producer FlB, there was a 50% decrease in the mycelial weights and in addition the quantity as well as the composition of the fat formed in the mycelia was changed (3). This was shown by the increase in the iodine number of the fat as compared with the controls, and subsequent analyses showed that this was due to the formation of a greater proportion of linoleic acid (16). Other synthetic naphthoquinones had similar effects (2).

When acetate was used as the carbon source, the above naphthoquinones had very little effect on the growth of the test organism, or on the quantity or degree of unsaturation of the fat formed. The quinones probably affect the enzyme systems which participate in glycolytic reactions (17). Under these circumstances, pyruvic acid was observed to accumulate in the culture medium to a much greater extent than in the controls (18).

The pigment lycopersin, obtained from Fusarium lycopersici, had an effect contrary to that of solanione and the synthetic naphthoquinones. A larger quantity of more saturated fat was formed when this pigment was added to the growth medium of the nonpigmenting variety of Fusarium vasinfectum (19).

It, therefore, appeared to be desirable to compare the possible effect of radicinin relative to fat formation with fusarial pigments. However, it was found that the addition of radicinin to the growth medium of FlB had no effect on the growth of the organism as judged by the mycelial weights, pH of the medium, or the residual glucose content of the culture filtrate.

To compare the behavior of radicinin with that of solanione and lycopersin in the synthesis of fat by FlB, the fats were isolated and analyzed (20). The results of these experiments, recorded in Table I, show that radicinin had no effect on the quantity or degree of unsaturation of the fats formed by FlB, when it was added to the growth medium in concentrations as high as 25 mg./l.

Radicinin thus presents a noteworthy contrast with the above-mentioned pigments.
TABLE I

<table>
<thead>
<tr>
<th>Conc. of radicinin (mg./l.)</th>
<th>Iodine value of fatty acids</th>
<th>Total lipides</th>
<th>Fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. / l.</td>
<td>%</td>
<td>Oleic</td>
</tr>
<tr>
<td>Control</td>
<td>115.6</td>
<td>5.90</td>
<td>10.5</td>
</tr>
<tr>
<td>1.0</td>
<td>117.9</td>
<td>5.90</td>
<td>9.7</td>
</tr>
<tr>
<td>5.0</td>
<td>113.2</td>
<td>5.84</td>
<td>11.7</td>
</tr>
<tr>
<td>25.0</td>
<td>115.6</td>
<td>5.81</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Values are expressed as the average of duplicate determinations. Fatty acids were obtained from 40 mats in each series.

Experimental

Microbiological

A culture of Sterad, obtained from the College of Agriculture, University of California, Berkeley, California, through the courtesy of Dr. W. C. Snyder, was used as the source of radicinin in these experiments. The stock cultures were maintained on potato dextrose agar medium. The organism was grown on potato dextrose liquid medium in 3-l. Fernbach flasks which contained 1 l. of potato dextrose liquid medium each.

Raulin-Thom medium (3) which had been adjusted to pH 5.5 by addition of alkali prior to sterilization was also found to be satisfactory for cultivating this organism for the purpose of isolating radicinin.

FlB, No. 5140, originally obtained from the Biologische Reichsantalt, Berlin-Dahlem, through the courtesy of the late Dr. H. Wollenweber, was used as a test organism for investigating the biological action of radicinin. The media described in previous papers from this laboratory were used (3).

Radicinin was isolated from the culture filtrate of Sterad by continuous extraction with ether. Twenty liters of the culture filtrate, which had been incubated for 4-5 weeks, was decanted from the mycelium, acidified to pH 2, and saturated with common salt. This was placed in three liquid-liquid extractors, and each extractor was connected to a boiling flask containing 400 ml. ether. After 4-5 days, the extraction was stopped, the ether cooled, and the crystalline radicinin filtered off. The ether was reused for later extractions. From 12 to 15 g. of crude pigment was thus obtained, and after one recrystallization from methanol (300 ml.) 7-10 g. of radicinin, m.p. 218-220°C., was obtained. After two more recrystallizations, the m.p. was constant at 220°C. (dec.), and the following analyses were obtained on a sample, which had been recrystallized five times from methanol.

Microanalyses were carried out by A. Sirotenko, formerly of this Department; Drs. Weiler and Strauss of Oxford, England; and Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.
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Anal. Calcd. for C_{12}H_{12}O_{5}: C, 61.01; H, 5.12; O, 33.87; CH_{3}—(C), 12.7 (2 groups); CH_{3}—O—, 0.0; mol. wt. 236.2;
found: C, 60.95; H, 5.38; O, 33.78; CH_{3}—(C), 11.9; OCH_{3}, 0.0; mol. wt. 240 (Rast); 236.5 (x-ray diffraction).^{8}
Radicinin belonged to the orthorhombic crystal system and had the space group R 2_{1}2_{1}2_{1},^{8} which agrees with the fact that it is optically active. [α]_{D}^{25} = −217.4° (c, 2.37 pyridine), −175.7° (c, 0.2 ethanol), −208° (c, 1.25 chloroform).

**Monoacetate**

One hundred milligrams of radicinin was added to 2 ml. acetic anhydride, and one drop of concentrated H_{2}SO_{4}, or two drops of pyridine was added to the mixture. Heat was evolved. After standing for 1 hr., the solution was poured into 30 ml. ice water with stirring. After recrystallization from methanol twice, the m.p. was 197°C.; yield, 80 mg.; [α]_{D}^{25} = −267.0° (c, 0.69 pyridine).

Anal. Calcd. for C_{14}H_{14}O_{6}: C, 60.43; H, 5.07; acetyl, 15.53; CH_{3}—(C), 16.3;
found: C, 60.31; H, 5.14; acetyl, 23.8 (alkaline hydrolysis), 20.4 (acid);^{2} CH_{3}—(C), 15.9.
Radicinin could be recovered from the acetate by dissolving the latter in a small quantity of 50% H_{2}SO_{4} and then pouring the solution into 5 vol. of cold water.

**2,4-Dinitrophenylhydrazone**

This derivative was prepared by dissolving 500 mg. radicinin in 100 ml. of ethanol and adding a freshly prepared solution of the reagent (21). Precipitation occurred on standing, and this crude material was purified by recrystallization from pyridine. After two recrystallizations, the m.p. was 235–238°C. (dec.). [α]_{D}^{25}, −11.0° (c, 0.10 pyridine).

Anal. Calcd. for C_{18}H_{16}N_{4}O_{8}: C, 51.93; H, 3.87; N, 13.46;
found: C, 52.10; H, 3.85; N, 12.92.

**Hydrogen Chloride Adduct**

One gram of radicinin was dissolved in 50 ml. of chloroform, and the solution was saturated with dry hydrogen chloride. After standing overnight, the chloroform was removed under reduced pressure and the residue recrystallized twice from methanol. An alternate method for isolating this derivative consisted in precipitating it from the chloroform solution by addition of petroleum ether (b.p. 30–60°C.); m.p. 156°C. (dec.).

Anal. Calcd. for C_{12}H_{13}O_{5}Cl: C, 52.85; H, 4.81; Cl, 13.00;
found: C, 53.11; H, 4.81; Cl, 13.07.

**Bromine Adduct**

One gram of radicinin was dissolved in 50 ml. of chloroform, and a solution of 0.25 ml. bromine in 1 ml. chloroform was added. After standing for an hour, the...

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^{8} In a preliminary communication (6), the space group was given as P 2_{1}2_{2}. When larger crystals of radicinin were examined, this space group had to be modified.
bromine color disappeared. The solution was washed with 2% NaHCO₃ solution and dried over Drierite. After evaporation of the chloroform and two recrystallizations from methanol, the melting point was constant at 151–2°C.

*Anal.* Calcd. for C₁₂H₁₂O₅Br₂: C, 36.39; H, 3.05; Br, 40.36; found: C, 36.35; H, 3.07; Br, 40.37.

When an excess of bromine was used in the above procedure, hydrogen bromide was evolved on standing, thus indicating that substitution was occurring.

**Acetate of Radicinin Hydrogen Chloride**

This was prepared in the same manner as the acetate of radicinin; m.p. 198°C.

*Anal.* Calcd. for C₁₃H₁₅O₆Cl: C, 53.43; H, 4.80; Cl, 11.27; found: C, 52.76; H, 4.37; Cl, 11.04.

**Acetate of Radicinin Dibromide**

This was prepared as above; m.p. 172°C. (dec.)

*Anal.* Calcd. for C₁₄H₁₄O₆Br₂: C, 38.38; H, 3.22; Br, 36.48; found: C, 38.44; H, 3.02; Br, 37.37.

**Radicinin Methyl Ether**

One gram of radicinin was dissolved in 100 ml. dry acetone, and 2 g. anhydrous potassium carbonate was added. The solution was heated under reflux, and 2 ml. dimethyl sulfate was added. Heating was continued for 12 hr. The reaction mixture was cooled and filtered, and the solid residue was washed with two 10-ml. portions of boiling acetone. The acetone solution was evaporated to a small volume and poured into water. The precipitate was filtered, dried, and sublimed in vacuo. The m.p. of samples of this derivative obtained in different runs was variable, and the best sample melted from 144 to 147°C.

*Anal.* Calcd. for C₁₃H₁₄O₅: C, 62.39; H, 5.64; OCH₃, 12.40; found: C, 62.08; H, 5.48; OCH₃, 11.97.

**Catalytic Hydrogenation**

One gram of radicinin was dissolved in 40 ml. of a mixture of glacial acetic acid and methanol (1:1) and shaken with 10 mg. 5% Pd on charcoal (Baker) or PtO₂ (Baker) for 8 hr. The pressure drop corresponded to the uptake of one mole of hydrogen. The solvent was evaporated and the residue recrystallized from methanol or isooctane. The m.p. was 156°C. This derivative, in contrast to radicinin, gave a positive test for a reactive methylene group.

When the above reaction mixture, using Pd on charcoal as catalyst, was shaken for 2 days, the pressure of hydrogen remained constant. After filtering off the catalyst, the solvent was removed and an oil remained. This product was extracted with petroleum ether on the steam bath and evaporated at room temperature. The product was still an oil, but, in contrast to radicinin and dihydroradicinin, it gave an amber color test with ferric chloride.

When platinum oxide was used as the catalyst in the same manner as above, an oil was again obtained, which was purified as above. This compound did not give a color test with ferric chloride. In this hydrogenation, 3.4 moles of hydrogen
was consumed. Under the same conditions, radicinin hydrogen chloride consumed 2.3 moles of hydrogen.

**Pyrolysis of Radicinin**

Radicinin (0.95 g.) was refluxed in 25 ml. diphenyl ether under an atmosphere of nitrogen purified by passage over hot copper gauze (800°C.). An air condenser was employed. The effluent gases were passed through two absorbers containing barium hydroxide solution. Titration of the barium hydroxide solution at the end of the experiment showed that 1.1 moles of CO₂ was absorbed. On cooling, a black residue separated. This product did not melt up to 300°C., and attempts to purify it were of no avail.

**Alkaline Hydrolysis**

One gram of radicinin was dissolved in 100 ml. 1% KOH and refluxed in a slow stream of nitrogen. The reflux condenser was fitted with a Kjeldahl trap, and the effluent gases were bubbled through a solution of 2,4-dinitrophenylhydrazine in 2 N HCl. After 6 hr. there was no further precipitation. The derivative was filtered off, dried, and weighed. Three hundred milligrams was thus obtained. After recrystallization from methanol twice, the m.p. was 158-61°C., and it did not depress the m.p. of an authentic sample of acetaldehyde-2,4-dinitrophenylhydrazone.

**Oxidation of Radicinin Dibromide**

One gram of dibromoradicinin was oxidized with KMnO₄ (21). A solid acid was obtained. After two recrystallizations from petroleum ether, the m.p. was 86-87°C. The literature value for the m.p. of α,β-dibromobutyric acid was 87°C. (7). All other possible acids melted at least 20°C. lower. The mixed m.p. with an authentic sample of α,β-dibromobutyric acid was 87-88°C. This compound liberated iodine when treated with sodium iodide in acetone.

**Anal.** Calcd. for C₆H₅Br₂O₂: C, 19.53; H, 2.46; Br, 64.99; Neut. Eq., 245.9; found: C, 19.81; H, 2.52; Br, 64.67; Neut. Eq., 250.
Alkali Fusion

Thirty grams potassium hydroxide was moistened with 1.2 ml. water and heated to 250°C. in a nickel crucible (100 ml.). Three grams of radicinin was added in small portions. Effervescence occurred, and there was an odor of caramel. The reaction mixture was heated rapidly to 300°C. while stirring with a thermometer enclosed in a copper well (8). The fused mixture was kept at 310°C. for 5 min., then cooled. When it started to become pasty, it was poured into an 800-ml. beaker half filled with ice and water. The solution was acidified with conc. HCl, the charred material filtered off, and the filtrate extracted with ether, until it no longer gave a positive Gibbs test (9). It was extracted four times with 50 ml. 1 M phosphate buffer each time. After washing the ether solution with a little water, it was extracted with four 50-ml. portions of 2% NaHCO₃ solution (22).

The phosphate buffer was acidified with 20 ml. 85% H₃PO₄ and extracted with three 50-ml. portions of ether. The bicarbonate solution was also acidified and re-extracted into ether. Each ether extract was dried over Drierite and evaporated to dryness under reduced pressure.

These degradation products were not identified, as less than one mg. of each was obtained.

Infrared Spectra

Unless otherwise specified, these were obtained as Nujol mulls on a Perkin-Elmer double-beam spectrophotometer.⁹

Ultraviolet Absorption Spectra

These were determined with a Beckman model DU spectrophotometer using 1.0-em. quartz cuvettes. Spectro grade methanol (Kodak) was used as the solvent unless otherwise specified.

Determination of Acetone

The conditions of growth of FIB and addition of the pigment were the same as reported (3). The determination of acetone was carried out according to Dal-Nogare et al. (23).

Fat Formation

These experiments were performed as described earlier (18, 20).

Comments

The structure that has been suggested for radicinin presents a rather unique arrangement of oxygen atoms in a molecule. An examination of a molecular model of this compound showed that in order to allow closure of the lactone ring and hydrogen bonding of the hydroxyl and carbonyl

⁹ We wish to thank Mrs. C. Jorgensen, formerly of Schering Corp., for having determined, and aided in the interpretation of the infrared absorption spectra.
groups, the three noncarbonyl oxygen atoms must be on the same side of the plane of the ring system of radicinin. Hence, even though position 7 is expected to be a labile asymmetric center, ring closure will proceed in only one direction, so that a single stereoisomer is formed.

Although radicinin is catalytically reducible, it differs from solanione in that it is not reversibly reducible. It, therefore, was not surprising that it did not participate in the dehydrogenation of fats, as did solanione (2). In addition, solanione was found to interact with reduced diphosphopyridine nucleotide in vitro (24), while radicinin did not. ¹⁰ There is also a noticeable contrast between the action of these two metabolites on the rate of dehydrogenation of isopropyl alcohol, but no precise mode of action on the enzyme systems involved can be suggested at present.

Summary

The conditions for the growth of Sterad and isolation of radicinin are described. The formula \( \text{C}_{12}\text{H}_{12}\text{O}_{5} \) was established for this compound by preparation and analyses of several derivatives. Degradation and spectrophotometric studies were employed to establish the molecular formula.

Studies on the mode of action of radicinin revealed that it caused an increase in the rate of dehydrogenation of isopropyl alcohol by FIB, but that it had no effect on the quantity or degree of desaturation of the fat synthesized by FIB.

References


¹⁰ Unpublished observation.
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