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IN VIVO BIOSYNTHESIS OF ISOPENTENYLACETOPHENONES IN *EUPATORIUM RUGOSUM*

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Key Word Index—*Eupatorium rugosum*; Compositae; Snakeroot; isopentenylacetophenones; dehydrotremetone; biosynthesis.

Abstract—The biosynthesis of dehydrotremetone in *Eupatorium rugosum* has been investigated by feeding radioactive precursors to intact plants. The carbon atoms of acetate-[1-^{14}C] and acetate-[2-^{14}C] were identified in dehydrotremetone by degradation of the molecule. From the pattern of labeling it was concluded that the acetophenone moiety was derived from acetate via the polyacetate pathway. From the incorporation of mevalonate it appeared that the furan ring and its side chain were formed from an isoprenoid compound. Potential aromatic intermediates were chemically synthesized and also fed to plants but only tremetone was found to be efficiently incorporated into dehydrotremetone. Neither 4-hydroxyacetophenone nor 4-hydroxy-3[isopenten-(2)-yl]-acetophenone were efficiently incorporated into dehydrotremetone.

INTRODUCTION

THE PROPOSED biosynthesis of dehydrotremetone (**1**) and tremetone (**2**), the toxic principles in snakeroot, is by isoprenylation of acetophenone.[1] The acetophenone moiety in turn can conceivably be formed by cyclization of a polyketide chain derived from the condensation of acetyl–CoA and malonyl–CoA. However, Bu'Lock *et al.*[2] have shown that a number of benzofurans isolated from *Stereum subpileatum* are not derived via a polyacetate but from aromatic amino acids or shikimic acid by side chain degradation.

This paper reports on the elucidation of the biosynthetic route in *Eupatorium rugosum* leading to the acetophenone moieties of **1** and **2**.

RESULTS AND DISCUSSION

Distribution of dehydrotremetone in E. rugosum

Initially various parts of *E. rugosum* plants, grown under controlled conditions in the greenhouse, were assayed for **1**. The highest amount occurred in the leaves of the plant (Table 1). Significantly less was found in the stem tissue with only trace amouns in the roots and seeds. The roots, however, contained significant amounts of eupariochromene

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[1] BOHLMANN, F. and GRENZ, M. (1970) *Chem. Ber.* **103**, 90.

[2] BU'LOCK, J. D., KAYE, B. and HUDSON, A. T. (1971) *Phytochemistry* **10**, 1037.

(6-acetyl-7-hydroxy-2,2-dimethylchromene) (2 mg/g fr. wt) and ripariochromene (6-acetyl-7-hydroxy-8-methoxy-2,2-dimethylchromene[3]) (1·9 mg/g fr. wt).[4] Furthermore, it appeared that the concentration of **1** increased as the plant matured.

TABLE 1. DISTRIBUTION OF DEHYDROTREMETONE IN *Eupatorium rugosum*

Tissue	Age (month)	Dehydrotremetone (mg/g fr. wt)
Leaf	3	0·127
Stem	3	0·074
Root	3	0·0001
Whole plant	1	0·1
Whole plant	4	0·123
Seed	—	0·0001

Incorporation of tracer compounds

The results of several experiments, in which 2-month-old plants were fed with radioactive compounds, are summarized in Tables 2–4. In Table 2 precursors, which were expected to be incorporated into the acetophenone moiety of **1** were tested. Either acetate-[1-^{14}C] or acetate-[2-^{14}C] was found to be much more efficiently incorporated into **1** than the aromatic acids phenylalanine and tyrosine. Cinnamic acid a potential precursor of the C_6–C_2 unit in **1**, by decarboxylation followed by α-oxidation, was a very poor precursor (Table 2). The utilization of acetate for the synthesis of **1** may be due to the selective incorporation of acetate into the furan ring and its side chain, with an aromatic precursor being utilized for the acetophenone moiety. This hypothesis, however, seemed unlikely because of the poor incorporation of the labeled aromatic compounds listed in Table 1. Furthermore, the degradation studies of **1** synthesized from either acetate-[1-^{14}C] or acetate-[2-^{14}C] (Table 3) also tended to eliminate this mechanism.

TABLE 2. INCORPORATION OF POSSIBLE PRECURSORS INTO DEHYDROTREMETONE

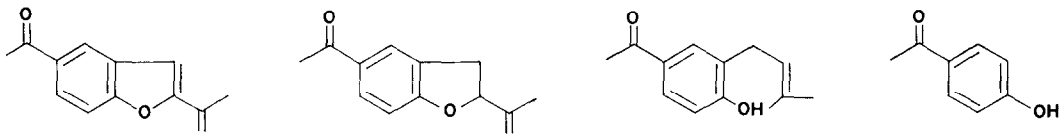
Precursor	Sp. act. (μCi/μmol)	Amount fed (μCi)	Dehydrotremetone Sp. act (μCi/μmol)	Dehydrotremetone Incorporation (%)
Acetate-[1-^{14}C]	56·0	40	$0{\cdot}46 \times 10^{-4}$	0·06
Acetate-[2-^{14}C]	56·0	40	$0{\cdot}49 \times 10^{-4}$	0·06
Phenylalanine-[2-^{14}C]	3·4	20	$0{\cdot}23 \times 10^{-6}$	0·0006
Tyrosine-[3-^{14}C]	1·5	15	$0{\cdot}43 \times 10^{-6}$	0·0004
Cinnamic acid-[3-^{14}C]	1·5	15	$0{\cdot}25 \times 10^{-6}$	0·0008
Glycine-[2-^{14}C]	57·0	10	$0{\cdot}94 \times 10^{-6}$	0·005

Origin of acetophenone portion of dehydrotremetone

An indication that the acetophenone moiety of **1** is derived either from a C_6–C_3 precursor or from acetate can be obtained from the distribution of the radioactivity from the carbon atoms of acetate in the compound (Table 3). With acetate-[1-^{14}C] as the precursor and the acetophenone moiety being derived via a polyketide precursor, carbon atoms

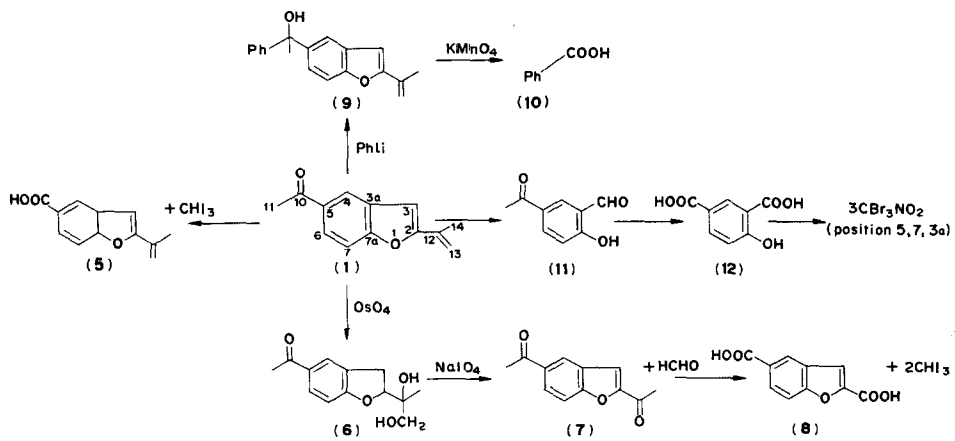
[3] ANTHONSEN, T. (1969) *Acta Chem. Scand.* **23**, 3305.
[4] LIN, T. J. and HEINSTEIN, P. unpublished results.

10, 6, 7a, and 4 of **1** (Scheme 1) should be labeled. Carbon atom 10 was isolated as benzoic acid (**10**) and its radioactivity was found to be in agreement with the calculated value (Table 3). Furthermore, only trace amounts of radioactivity are found in carbon atoms 11, 5, 7 and 3a. However, if acetate-[2-^{14}C] is used, then carbon atoms 11, 5, 7, and 3a of **1** should be labeled. Carbon atom 11 was isolated as iodoform (Scheme 1) and its radioactivity was found to be in agreement with the calculated value (Table 3). Positions 5, 7 and 3a were isolated together as tribromonitromethane (Scheme 1) and also contained radioactivity close to the theoretical value (Table 3). In addition, only a small amount of radioactivity was located in carbon atom 10. The observed radioactivity associated with compound **11** and **8** showed the biggest deviation from the calculated value (Table 3).



(1) (2) (3) (4)

However this was not entirely unexpected since it appeared that **1** was derived from acetate via two separate pathways, the aromatic portion via a polyacetate and part of the furan ring and its side chain via the isoprenoid pathway. Therefore, a slightly different utilization of acetate as precursor in the two pathways could account for these discrepancies.



Scheme 1. DEGRADATION OF DEHYDROTREMETONE (**1**).

Although attempted several times, it was not possible to accumulate sufficient radioactive **1** from either phenylalanine-[2-^{14}C] tyrosine-[3-^{14}C] or cinnamic acid-[3-^{14}C] to carry out degradative studies. Nevertheless, if the radioactive acetate fed to the plants was incorporated via the shikimic acid pathway considerably more randomization of the labeled carboxyl- or methyl-carbon of acetate in **1** would have been expected.

Origin of furan ring and its side chain

This portion of **1** has been postulated to be derived from an isoprenoid unit[1,2] and, therefore, a number of isoprenoid compounds or compounds which can be converted to isoprene units were fed to plants. As is usually observed in intact plant feeding experiments

MVA and the two pyrophosphorylated isoprene compounds IPP and DMAPP were poor substrates (Table 4). In the latter two compounds the poor incorporation is most probably caused by impermeability. The same might also be true for MVA. However, it has been shown that sucrose can stimulate incorporation of MVA into mono- and sesquiterpenes[5] and the increase is thought to be due to an increased supply of energy at the site of MVA utilization. MVA incorporation into **1** was stimulated by sucrose (Table 3), however 3-hydroxy-3-methyl-glutaric acid, a precursor of MVA in the isoprenoid pathway, was found to be more efficiently incorporated into **1**.

TABLE 3. DISTRIBUTION OF ^{14}C FROM ACETATE-[1-^{14}C] AND ACETATE-[2-^{14}C] IN DEHYDROTREMETONE

Compound	Position*	Acetate-[1-^{14}C] Calculated† (dpm/μmol)	Acetate-[1-^{14}C] Found (dpm/μmol)	Acetate-[2-^{14}C] Calculated† (dpm/μmol)	Acetate-[2-^{14}C] Found (dpm/μmol)
1	A11		156·0		104·0
5	A11–11	156·0	154·0	89·2	90·8
Iodoform	11	0	0·4	14·8	13·0
6	A11	156·0	156·0	104·0	104·0
7	A11–13	156·0	154·2	89·2	92·6
Formaldehyde	13	0	1·4	14·8	12·6
8	A11–11,13	156·0	146·1	74·4	62·2
Iodoform	11,13	0	4·4	14·8	15·2
10	10	26	21·4	0	3·6
11	3 to 11	125	116·4	59·2	43·3
1	A11		41·2		72·8
11	3 to 11	34·6	24·0	41·6	43·5
Bromopicrin		0	0·04	10·4	6·2

* Refers to Scheme 1.

† The calculated values were based on isolated dehydrotremetone with the assumption that the acetophenone portion of the compound was derived via the polyacetate pathway and the furan portion from an isoprenoid compound.

TABLE 4. INCORPORATION OF ISOPRENOID PRECURSORS INTO DEHYDROTREMETONE

Compounds	Sp. act (μCi/μmol)	Amount fed (μCi)	Dehydrotremetone Sp. act. (μCi/μmol)	Dehydrotremetone Incorporation (%)
DL-Mevalonate-[2-^{14}C]	10·3	20	0	0
DL-Mevalonate-[2-^{14}C] + Sucrose*	10·3	20	$2{\cdot}05 \times 10^{-4}$	0·001†
DL-3-Hydroxy-3-methyl-glutaric acid-[1-^{14}C]	1·6	4	$0{\cdot}44 \times 10^{-5}$	0·08†
Isopentenyl pyrophosphate-[1-^{14}C]	0·09	5	$0{\cdot}5 \times 10^{-7}$	0·0008
Dimethylallyl pyrophosphate-[1-^{14}C]	0·14	5	$0{\cdot}3 \times 10^{-7}$	0·0004

* 10 μmoles of sucrose.

† Corrected for one isomer.

When **1**, synthesized from MVA-[2-^{14}C] was degraded to **11**, only 6·4% of the radioactivity originally found in **1** was recovered in **11**. This indicated that MVA gives rise preferentially to the furan ring and its side chain in dehydrotremetone.

[5] CROTEAU, R., BURBOTT, A. J. and LOOMIS, W. D. (1972) *Phytochemistry* **11**, 2937.

Utilization of potential aromatic intermediates

It has been inferred that in the pathway leading to dehydrotremetone, 4-hydroxy-acetophenone (**4**) is alkylated by a pyrophosphorylated isoprenoid to give 4-hydroxy-3(isopenten-(2)-yl)acetophenone (**3**), which undergoes cyclization to tremetone, followed by oxidation to dehydrotremetone. Compounds **2**, **3** and **4** were tritiated and fed to young plants. The radioactivity of the isolated dehydrotremetone upon recrystallization to constant specific activity showed that **2** was efficiently incorporated (Table 5). However the dilution of the radioactivity of **2** was found to be rather high ($6{\cdot}2 \times 10^5$) for a one step precursor-product relationship. On the other hand, in view of the significant amounts of **2** in *E. rugosum* plants this dilution of specific radioactivity appeared to be reasonable. Nevertheless from these results it was not possible to establish that tremetone is a precursor of dehydrotremetone or if both compounds were derived from a common intermediate.

TABLE 5. INCORPORATION OF POTENTIAL AROMATIC INTERMEDIATES INTO DEHYDROTREMETONE

Compound	Sp. act. (μCi/μmol)	Amount fed (μCi)	Dehydrotremetone Sp. act. (μCi/μmol)	Dehydrotremetone Incorporation (%)
4-Hydroxyacetophenone	26·7	9·0	$0{\cdot}11 \times 10^{-4}$	0·04
4-Hydroxy-3[isopenten-(2)-yl] acetophenone	4·07	50·3	$0{\cdot}06 \times 10^{-4}$	0·004
Tremetone	8·0	4·0	$0{\cdot}13 \times 10^{-4}$	0·11

Compound **3** showed little incorporation into dehydrotremetone (Table 5) and repeated attempts failed to locate **3** in extracts of the plants. From this, together with the dilution factor of **3** ($6{\cdot}8 \times 10^5$), it appeared either that compound **3** is not a direct precursor of dehydrotremetone or that it was impossible for the compound to overcome membrane impermeabilities and reach the site of the synthesis of dehydrotremetone.

Although the incorporation of **4** into dehydrotremetone was 10 times more than **3**, its specific activity was much more diluted ($2{\cdot}4 \times 10^6$) than this compound. Again the presence of **4** in the ketone fraction obtained from the plants extract could not be verified. Therefore, excluding possible problems in crossing membranes, compound **4** appeared not to be a precursor of dehydrotremetone.

Conclusion

The results obtained with feeding experiments confirmed that dehydrotremetone is formed biosynthetically from acetate via a polyketide intermediate which gives rise to the acetophenone moiety and from an isoprenoid unit which gives rise to the furan ring and its side chain. Disregarding permeability problems, it appeared that neither 4-hydroxy-3[isopenten-(2)-yl] acetophenone nor 4-hydroxyacetophenone are efficient precursors of the compound. Consequently the proposed biosynthetic route leading from a polyacetate intermediate to an acetophenone compound, followed by alkylation of an aromatic intermediate, appeared unlikely. Although tremetone was efficiently incorporated into dehydrotremetone and could be considered a precursor of this compound, the synthesis of both compounds from a common intermediate cannot be excluded.

EXPERIMENTAL

Extraction and characterization of dehydrotremetone and tremetone. Eupatorium rugosum Houtt plants and seeds were initially collected in McCormick woods, West Lafayette, Indiana during May to September; subsequently plants were grown in the greenhouse. Plant material was extracted with 80% MeOH in a Waring Blender for 1 min at maximum speed. The slurry was percolated with MeOH and the combined extracts evaporated to a semi-solid consistency. The crude residue (30 g) was dissolved in 30 ml $CHCl_3$, mixed with 10 g of silicic acid and after evaporation of the solvent applied to a silicic acid column (4 × 100 cm) and eluted with C_6H_6 (3 l.). The eluate after evaporation gave a red syrup termed the ketone fraction which was practically free of chlorophyll and amounted to 0·012% fr. wt. The ketone fraction (10 g) was dissolved in C_6H_6 (5 ml) and added to a silica gel column (4 × 60 cm) and eluted with C_6H_6. After an initial yellow band, a blue fluorescent fraction was eluted which was identified as dehydrotremetone (**1**) by TLC on silica gel using MeOH–C_6H_6 (1:49). Pure **1** (500 mg) was obtained after crystallization from C_6H_6 and recrystallization (3 ×) from hexane. M.p. 87–89°; UV λ_{max}^{MeOH}: 254 nm (ε 40000), 279·2 nm (ε 19800), 292·5 nm (ε 15200); IR identical to literature;[6] PMR ($CDCl_3$) δ 2·18 (3 H, *s*, C<u>H</u>$_3$–), 2·66 (3 H, *s*, C<u>H</u>$_3$CO), 5·30 and 5·85 (1 H each, *m* =C<u>H</u>$_2$), 7·51 (1 H, *d*, *J* 10 Hz, H-7), 8·02 (1 H, *d*, *d*, *J* 2, 10 Hz, H-6), 8·25 (1 H, *J* 2 Hz, H-4) and 6·75 (1 H, *s*, H-3); MS *m/e* (rel. intensity): 200 (M^+, 45), 185 (100), 157 (36), 129 (17), 43 (1). Routine quantitative determinations of dehydrotremetone involved grinding the plant material in an Omni-Mixer with MeOH, refluxing with MeOH for 3 hr, concentration *in vacuo* and extraction of the residue 3× with $CHCl_3$. The combined $CHCl_3$-extracts were concentrated, aliquots spotted on a silica gel TLC plates and developed with MeOH–C_6H_6 (1:45). Dehydrotremetone was detected by UV, removed from the plates, eluted with MeOH and the soln adjusted to 25 ml with MeOH. Since a plot of A at 292·5 nm vs [dehydrotremetone] followed Beer's law, this soln was used to assay the compound.

From the mother liquors from the crystallization of dehydrotremetone, the semicarbazone of tremetone (**2**) was obtained after addition of semicarbazide. The semicarbazone of **2** was recrystallized once from 95% EtOH and hydrolyzed by shaking with 3 N H_2SO_4 and 100 ml petrol (60–80°) for 3 days at 20°. After separation of the 2 layers, the acid portion was extracted with 50 ml petrol (60–80°), the petrol. dried and evaporated. The residue dissolved in C_6H_6 was chromatographed on a silicic acid column (2 × 30 cm) and eluted with C_6H_6 which gave **2** identified by silica gel TLC using MeOH-benzene (1:49). After evaporation of the C_6H_6, 2 g pure **2** was obtained as an oily liquid. M.p. (semicarbazone) 220°; UV λ_{max}^{MeOH}: 229 nm (ε 11900), 280 nm (ε 13000), and 298 nm (ε 11300); IR ($CHCl_3$): 3·34, 5·71, 5·95, 6·17, 6·23, 6·66, 6·90, 7·24, 7·41, 7·69, 8·00, 8·01, 8·77, 10·20, 10·30, and 10·87 μm; PMR ($CDCl_3$) δ: 1·70 (3 H, *d*, *J* 1 Hz, C<u>H</u>$_3$–), 2·33 (3 H, *s*, C<u>H</u>$_3$CO), 2·99, 3·13 (1 H each, *m*, C<u>H</u>$_2$), 4·76, 4·96 (1 H each, =C<u>H</u>$_2$), 5·06 (1 H, *t*, *J* 8 Hz, H-2), 6·58 (1 H, *d*, *J* 9 Hz, H-7).

Radioactive compounds. The following compounds were commercially purchased: DL-mevalonic acid-[2-^{14}C], acetate-[1-^{14}C], acetate-[2-^{14}C], phenylalanine-[2-^{14}C], glycine[2-^{14}C], and DL-3-hydroxy-3-methyl$_3$-glutaric acid-[1-^{14}C] from Amersham Searle Corporation; tyrosine[3-^{14}C] from Tracer Lab; cinnamic acid-[3-^{14}C] was a gift from Dr. H. G. Floss. Isopentenyl pyrophosphate-[1-^{14}C] and Dimethylallyl pyrophosphate[1-^{14}C] were synthesized as reported previously.[7,8] 4-Hydroxy-3[isopenten-(2)-yl]acetophenone (**3**) was prepared by isoprenylation of 4-hydroxyacetophenone. To 2 g of NaH (50% in oil) was added 10 g of **4** in 10 ml dry C_6H_6. After reflux ing for 30 min at 100°, a soln of 6 g of dimethylallylbromide[9] in 10 ml of dry C_6H_6 was added dropwise. The reaction mixture was refluxed for 6 hr, kept for 16 hr at 20° and filtered. The filtrate was washed with 10 ml of H_2O and conc., yielding 5 g of a crude oil. 3 compounds were isolated after chromatography on a silicic acid column (2 × 30 cm) and elution with C_6H_6. One of these was identified as **3** by comparison of its properties with the literature.[1,10] Tremetone, 4-hydroxy-3[isopenten-(2)-yl]acetophenone and 4-hydroxyacetophenone were tritiated according to Long *et al.*[11] and purified by repeated TLC on silica gel using MeOH–C_6H_6, (2:98), or MeOH–C_6H_6 (3:97). Compound **4** was purified by repeated re-crystallization to constant sp. act.

Feeding experiments. Plants used in these experiments were grown from seed in a greenhouse for 2–3 months. Thereafter the plants were moved to a growth chamber for feeding radioactive compounds and maintained at 25° by day and 15° by night. A 24 hr cycle consisted of a 16 hr day period under 4 wide-spectrum Gro-Lux lights and 8 ITT 40 W lights, maintained 1 m above the plants, and an 8 hr night period. Humidity was maintained at 90%. The wick feeding method was used, in which two strands of non-mercerized cotton were threaded through the stem of the plant and immersed at both ends into 1–2 ml soln containing the radioactive precursor. The plants were harvested after 2–3 days and dehydrotremetone extracted as before. Unlabeled compound was added and the resulting mixture recrystallized to constant sp. act.

Degradation of dehydrotremetone (**1**). The reactions are summarized in Scheme 1 and included the following compounds and reactions. *2-Isopropenyl-5-carboxylbenzofuran* (**5**): to a soln of **1** (20 mg) in 2 ml of dioxane and 0·5 ml of 10% NaOH an I_2-KI soln (2·5 g I_2 and 5 g KI in 200 ml of H_2O) was added dropwise with shaking

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[11] LONG, M. A., GARNETT, J. L. and VINING, R. F. W. (1972) *J. Am. Chem. Soc.* **94**, 8632.

until the reaction mixture remained dark in color. The reaction was maintained at 60° for 10 min. Thereafter a drop of 10% NaOH was added, decolorizing the soln, followed by 10 ml of H_2O, which precipitated CHI_3. The iodoform was sublimed and recrystallized from 95% EtOH. The remaining alkaline reaction mixture was acidified with 10% HNO_3, decolorized with 10% $Na_2S_2O_3$, and extracted 3 × with Et_2O (10 ml). After removing most of the solvent **5** was purified by chromatography on silica gel (1 × 10 cm) using MeOH–C_6H_6 (1:49) and yielded 10 mg after recrystallization from benzene, M.p. 157° (sublimation). UV, IR, PMR and MS were in agreement with structure **5**. 2-(1-*Hydroxy*-1-*methyl-ethanolyl*)-5-*acetylbenzofuran* (**6**): to a soln of 30 mg of **1** was added 50 mg of OsO_4, which was stirred in the dark for 12 hr then hydrolyzed with 10 ml of aq. KOH (5 g/125 ml) and mannitol (300 mg) and **6** extracted with $CHCl_3$.[12] Yield: 25 mg after recrystallization from benzene. M.p.: 117–119°, UV, IR, PMR and MS were in agreement with structure **6**. Since all the carbons of **1** were recovered in **6**, this reaction was used as a radioactive purity test of **1**.

2,5-*Diacetylbenzofuran* (**7**) to 68 mg of **6** in 2 ml of dioxane–H_2O (1:1), 200 mg of $NaIO_4$ was added.[12] After 2 hr at 20° Et_2O extraction yielded **7**, which after recrystallization from MeOH weighed 30 mg. M.p.: 145–145°, UV, IR, PMR and MS were in agreement with structure **7**. The aq reaction mixture was added to a filtered soln of 100 mg of dimedone in 10 ml of H_2O. After 2 hr at 20° formalin dimedone was collected and recrystallized from 75% MeOH. Its IR (KBr) was identical to that of authentic formalin dimedone. 2,5-*Dicarboxylbenzofuran* (**8**) **7** was subjected to the iodoform reaction and CHI_3 isolated as above. Attempts to isolate **8** failed, its sp. act. was therefore obtained by difference from **7** and CHI_3. *Benzoic acid* (**10**): **1** (100 mg) in dry Et_2O was added to a phenyllithium soln, prepared by the addition of 0·3 ml of bromobenzene to 30 mg Li in 2 ml of dry Et_2O, at 20°.[13] After 5 hr at 20°, H_2O was added and **9** extracted with Et_2O, which was purified on silica gel (1 × 10 cm) and elution with C_6H_6. After crystallization from C_6H_6, **9** was refluxed with $KMnO_4$ (410 mg) in H_2O for 16 hr. **10** was extracted with Et_2O after acidification and sublimed. Its IR was identical compared with that of an authentic sample. 2-*Hydroxy*-5-*acetylbenzaldehyde* (**11**): **1** (100 mg) in 3 ml of 75% dioxane in H_2O was treated with 0·1 ml of C_5H_5N, 0·2 ml of cyclohexane containing 20 mg of OsO_4 and 100 mg of $NaIO_4$, and stirred for 3 days in the dark at 20°. The reaction mixture was distilled and the residue applied to a silica gel column (1 × 10 cm), which was eluted with MeOH–C_6H_6 (1:49). The first compound eluted was found to be **7**. The second band consisted of **11**. It was recrystallized from C_6H_6 and IR, PMR and MS were in agreement with structure **11**. *Bromopicrin*: **11** (15 mg) was oxidized for 12 hr with 50 mg of $KMnO_4$ in 10 ml of H_2O at 20°. After acidification, the mixture was extracted with Et_2O, the solvent evaporated and the residue treated at 0° with 0·3 ml of a mixture of conc HNO_3 and conc H_2SO_4 (1:1). The reaction was stirred for 2 hr at 0°, thereafter 0·3 ml of conc HNO_3 was added and the reaction mixture was heated at 100° for 1 hr. Picric acid[14] was extracted with Et_2O after the addition of ice H_2O. The isolated picric acid behaved identically to authentic picric acid on silica gel TLC using CH_2Cl_2–MeOH–NH_4OH (80:20:3). A soln of picric acid and $Ba(OH)_2$ (0·2 g) in H_2O was cooled to 0° and treated dropwise with 0·18 ml of Br_2 and 1 g of $Ba(OH)_2$ in 10 ml of H_2O. The soln was stirred at 0° for 1 hr. Bromopicrin was isolated by steam distillation and Et_2O extraction of the distillate.

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