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45. Shinsaku Natori,*1 Yuko Inouye (née Kumada),*1,2 and Hidejiro Nishikawa*3 : The Structures of Mompain and Deoxyhelicobasidin and the Biosynthesis of Helicobasidin, Quinonoid Metabolites of *Helicobasidium mompa* TANAKA.*4

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The structure of mompain, a metabolite of *Helicobasidium mompa*, was established as 2,5,7,8-tetrahydroxy-1,4-naphthoquinone (IIa). Deoxyhelicobasin was isolated and the structure was elucidated as Ib. Biosynthesis of helicobasidin (Ia) was studied and its isoprenoid origin has been clarified.

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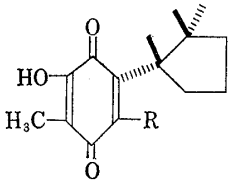
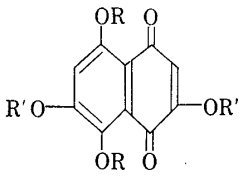
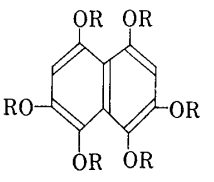
In the previous paper[1] the structure of helicobasidin (Ia), one of the two major metabolites of *Helicobasidium mompa* TANAKA (ムラサキモンパ病菌) (Tremellales, Basidiomycetes) was reported. In this paper the structures of the other major pigment, mompain (IIa), and a newly isolated minor constituent, deoxyhelicobasidin (Ib), will be discussed.*4 Furthermore biosynthesis of the pigments was studied and the results so far obtained will be reported.



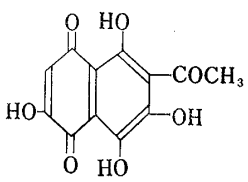
Ia : R=OH
b : R=H



IIa : R, R'=H
b : R, R'=CH_3CO
c : R=H, R'=CH_3
d : R=CH_3CO, R'=CH_3
e : R, R'=CH_3



III : R=CH_3CO



IV

Mompain, first isolated by Nishikawa,[2] exists in the mycelium along with helicobasidin and is the major pigment of the culture filtrate. The typical deep violet color of the mold is chiefly due to the presence of this pigment.

Mompain (IIa) comes as deep red leaflets of m.p. >300° (gradually decomposes at about 300° to 360°). It sublimes over 200°, shows dark green coloration with ferric chloride and reddish violet with magnesium acetate. It forms blue-violet precipitates with lead acetate. The aqueous solution of IIa shows a series of color change as the pH of the solution is varied; in acidic the solution shows orange color, at neutral red, and in alkaline deep violet. The color in alkaline solution is decolorized by the addition of sodium dithionite. The molecular formula of IIa was established as $C_{10}H_6O_6$ by elemental analyses and a mass spectrum (M^+ 222).

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*3 Shimouma-3-chome, Setagaya-ku, Tokyo (西川英次郎).
*4 Preliminary accounts of a part of this paper have been published : This Bulletin, **13**, 633 (1965).
1) S. Natori, H. Ogawa, H. Nishikawa : This Bulletin, **12**, 236 (1964).
2) H. Nishikawa : Agric. Biol. Chem. (Tokyo), **26**, 696 (1962).

Mompain gave tetraacetate (IIb), m.p. 176~179°, and dihydrohexaacetate (III), m.p. 229~232°. Dimethyl ether (IIc), m.p. 260~262°, was obtained by the methylation either with ethereal diazomethane, with methanolic hydrogen chloride, or with dimethyl sulfate and potassium carbonate in acetone. The dimethyl ether was further acetylated to give dimethyl ether diacetate (IId), m.p. 249~250°. Methylation of IIa with dimethyl sulfate and potassium carbonate or with methyl iodide and silver oxide afforded, though in a poor yield, tetramethyl ether (IIe), m.p. 169~171°, along with the dimethyl ether (IIc).

The ultraviolet absorption of IIa and the derivatives, especially that of the acetate (IIb), λ_{max}^{EtOH} mμ: 248, 352, clearly indicated that IIa has 1,4-naphthoquinone nucleus[3] (Fig. 1). pKa of IIa, determined by spectroscopic method, showed 4.5, 7.5, and >11.0, indicating the presence of one strongly acidic group and not less than two weakly acidic groups.[4] The molecular formula, the formation of the derivatives, and the IR and NMR spectra showed the presence of four hydroxyl groups on the nucleus and the absence of other substituents.

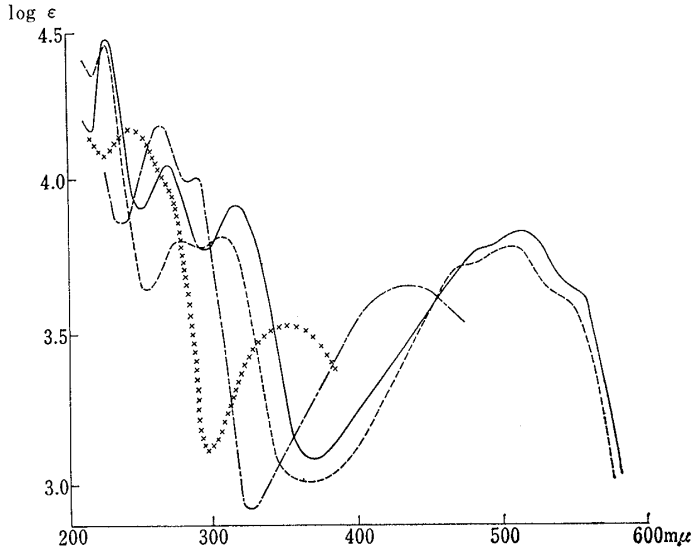


Fig. 1. Ultraviolet Spectra of Mompain and the Derivatives (EtOH solution)

——— mompain (IIa)
××××× tetraacetate (IIb)
– – – – – dimethyl ether (IIc)
—·— ·— tetramethyl ether (IIe)

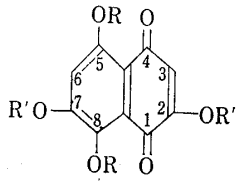
There are eight isomers of tetrahydroxy-1,4-naphthoquinones as follows: 2,3,5,6- (A), 2,3,5,7- (B), 2,3,5,8-(=5,6,7,8-)(C), 2,3,6,7- (D), 2,5,6,7- (E), 2,5,6,8- (F), 2,5,7,8- (G), and 2,6,7,8-tetrahydroxy-1,4-naphthoquinones (H). Among these isomers B and C are known compounds in literatures as spinochrome B[5] (spinochrome N[6]) and spinazarin[7] respectively and their properties are different from IIa. Tetramethyl ethers of E and H were synthesized by the authors[8] and have been proved to be different from IIe. Formation of the dimethyl ether (IIc) and the ultraviolet absorption[3] of IIa, λ_{max}^{EtOH} mμ: 228, 272, 318, 486, 517, 554, and IIc, λ_{max}^{EtOH} mμ: 227, 277, 308, 475, 507, 544 (Fig. 1), suggested that IIa is a naphthazarin derivative, in which the both peri positions are occupied by hydroxyl groups. These findings leave the structures F and G as the possible structure of IIa.

At this stage of the work, the structure of spinochrome A (M),[9] was revised to IV by Scheuer, *et al.*[10,11] The Hawaiian workers treated IV with methanolic hydrogen

3) R. A. Morton, W. J. Earlam: J. Chem. Soc., **1941**, 159; A. K. Macbeth, *et al.*: *Ibid.*, **1935**, 325; **1937**, 1597; **1939**, 878.
4) Lord Todd, *et al.*: *Ibid.*, **1964**, 49.
5) J. Gough, M. D. Sutherland: Tetrahedron Letters, **1964**, 269.
6) J. Smith, R. H. Thomson: J. Chem. Soc., **1961**, 1008.
7) R. Kuhn, K. Wallenfels: Ber., **75**, 407 (1942); C. Kuroda: Proc. Imp. Acad. Japan, **15**, 226 (1939).
8) S. Natori, Y. Kumada: This Bulletin, **13**, 1472 (1965).
9) C. Kuroda, M. Okajima: Proc. Japan Acad., **36**, 429 (1960); M. Okajima: Sci. Papers Phys. Chem. Research, **53**, 356 (1959).
10) C. W. J. Chang, R. E. Moore, P. J. Scheuer: J. Am. Chem. Soc., **86**, 2959 (1964).
11) *Idem*: Tetrahedron Letters, **1964**, 3557; I. Singh, R. E. Moore, C. W. J. Chang, P. J. Scheuer: J. Am. Chem. Soc., **87**, 4023 (1965).

chloride and obtained dimethyl ether of a tetrahydroxy-1,4-naphthoquinone, m.p. 235~236°. The spectroscopic properties, especially the NMR spectrum of the dimethyl ether, suggested that it must be either that of F or G, in which the former was ruled out by the comparison with the synthetic specimen, m.p. 295~296°. From these results the structure (Ⅳ) has been put forward to spinochrome A (M) by Scheuer, *et al.*[10] Although the some properties, especially NMR spectra,*4 showed that Ⅱc must be identical with the dimethyl ether derived from Ⅳ, discrepancy of the melting points urged us the re-examination of the deacetylation reaction. Spinochrome A (M) (Ⅳ), kindly supplied by Dr. Okajima,*5 was treated with conc. sulfuric acid to afford the desacetyl compound, and the crude reaction product was proved to be identical with Ⅱa by thin-layer chromatography. Methylation of the crude reaction product with diazomethane and purification by chromatography on $CaHPO_4$ afforded dimethyl ether of desacetylspinochrome A (M), m.p. 260~262°, which was actually identical with mompain dimethyl ether (Ⅱc) in every respect (m.p., a mixed fusion, thin-layer chromatography in two solvent systems and IR spectra).*6 Thus the structure of mompain was unequivocally established as 2,5,7,8-tetrahydroxy-1,4-naphthoquinone (Ⅱa=G).*1

Table I. Nuclear Magnetic Resonance Spectra of Mompain and the Derivatives



	Solvent	C_3- and C_6-H	C_2- and C_7-OH or OCH_3	C_5- and C_8-OH, OCH_3, or $OCOCH_3$
Mompain (Ⅱa)	DMSO	6.31(2H)	——	ca. 13.3
Dimethyl ether (Ⅱc)	$CDCl_3$	6.40(2H)	3.97(6H)	12.73(1H) 13.16(1H)(br.)
Dimethyl ether diacetate (Ⅱd)	〃	5.98(1H) 6.92(1H)	3.87(3H) 3.95(3H)	2.45(6H)
Tetramethyl ether (Ⅱe)	〃	5.96(1H) 6.78(1H)	3.82(3H) 3.87(3H)	3.96(6H)

δ values in p.p.m. from TMS. All signals are singlets.

NMR spectra of Ⅱa and the derivatives (Ⅱc, d, e), shown in Table I, clearly indicated the symmetrical disposition of hydroxyl groups in Ⅱa and Ⅱc and the structure (G) only suffices the condition in the eight isomers.*4,10 However the recent finding

*5 Professor C. Kuroda and Dr. M. Okajima[12] have now accepted the revision of the structure by Scheuer, *et al.*[10]

*6 (Note Added in Proof): After the submission of this paper for publication N. N. Gerber and B. Wieclawek (J. Org. Chem., **31**, 1496 (1966)) reported the isolation of 5,8-dihydroxy-2,7-dimethoxynaphthoquinone, m.p. 275~276°, from a strain of nonsporulating *Streptomyces*, no. 12396, and established the identity with the desacetyl compound from spinochrome M, referring our preliminary communication.*4
Quite recently 2,5,7,8-tetrahydroxynaphthoquinone itself has been isolated from sea urchin (Genus *Echinothrix*) along with a lot of related compounds (R. E. Moore, H. Singh, P. J. Scheuer : J. Org. Chem., **31**, 3645 (1966)) and chemistry and spectroscopic data of these compounds have also been reported (R. E. Moore, P. J. Scheuer : *Ibid.*, **31**, 3272 (1966); R. E. Moore, H. Singh, C. W. J. Chang, P. J. Scheuer : *Ibid.*, **31**, 3638 (1966); D. Becher, C. Djerassi, R. E. Moore, H. Singh, P. J. Scheuer : *Ibid.*, **31**, 3650 (1966)).

12) C. Kuroda, M. Okajima : Proc. Japan Acad., **40**, 836 (1964).

by X-ray analysis of cordeauxiaquinone[13] may suggest the possibility that the compound exist in the form of V in a crystalline state.

Mass spectrum of IIa (Fig. 2) will be explained by the fragmentations shown in Chart 1 as was elucidated by Bowie, *et al.*[14]

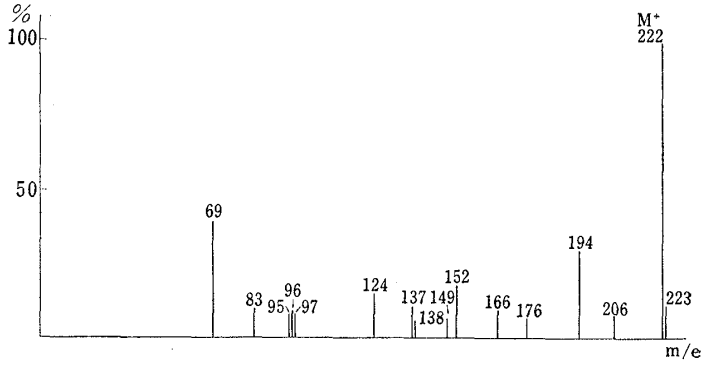


Fig. 2. Mass Spectrum of Mompain (IIa)

Ions having an abundance greater than 5% of that of the base peak are recorded.

Mompain is assumed to be originated from one acetate plus four malonate units and the structure corresponds to the 8-hydroxy derivative of flaviolin, the metabolite of *Aspergillus niger* gr.[15]

After our preliminary communication of this work,*4 Thomson synthesized 2,5,7,8-tetrahydroxy-1,4-naphthoquinone and the synthetic specimen has been proved to be identical with mompain.[16]

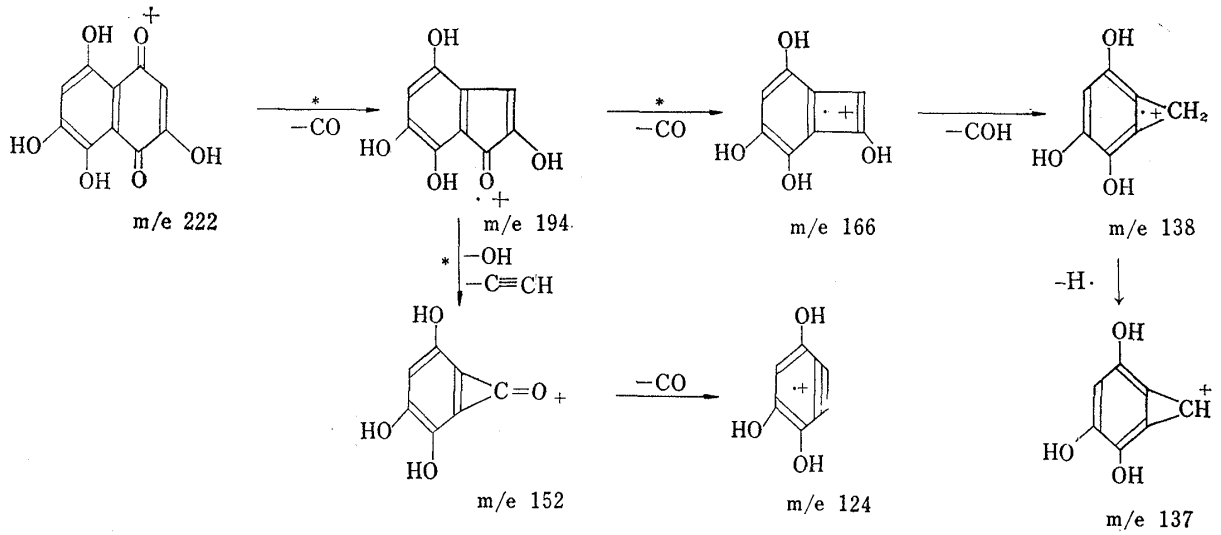
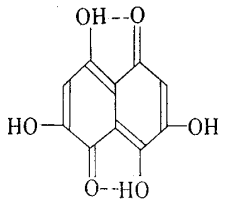


Chart 1. Fragmentation of Mompain

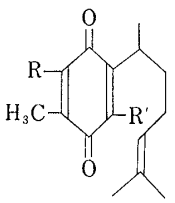
The reaction with asterisk has been confirmed by the respective metastable peak.

In the previous paper[1] we have mentioned that the crude sample of helicobasidin (Ia) contains a small amount of a closely related compound. Now the compound has been isolated by the chromatography through acid-washed silica gel as yellow needles (Ib) of m.p. 194~195°, $[\alpha]_D$ −186° ($CHCl_3$). Elemental analysis shows a molecular formula, $C_{15}H_{20}O_8$, having one less oxygen atom than Ia. Ultraviolet absorption of Ib shows the maxima at 274 and 404 mμ (log ε, 4.11, 3.02), which are shorter than those of Ia (297, 430 mμ) and agree well with those of 2-hydroxy-3,6-dialkylbenzoquinone

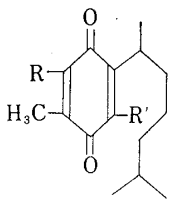
13) M. Fehlmann, A. Niggli: Helv. Chim. Acta, **48**, 305 (1965).
14) J. H. Bowie, D. W. Cameron, D. H. Williams: J. Am. Chem. Soc., **87**, 5094 (1965).
15) B. D. Astill, J. C. Roberts: J. Chem. Soc., **1953**, 3302; J. E. Davies, F. E. King, J. C. Roberts: *Ibid.*, **1955**, 2782.
16) Prof. R. H. Thomson: Private communication; A. C. Baillie, R. H. Thomson: J. Chem. Soc., (C), **1966**, 2184.



V



VIa : R=OH, R′=H
b : R=H, R′=OH
c : R=OH, R′=OH



VIIa : R=OH, R′=H
b : R=H , R′=OH

(270, 406 mμ).[1] Infrared absorptions of Ib, 3275 (OH), 1664, 1633, 1619 (sh), 1594 (C=O, C=C), also agree well with those of dialkylhydroxybenzoquinone.[1] Thus the compound (Ib) is quite probable to be deoxyhelicobasidin. NMR spectrum of Ib clearly demonstrated the assumption (Table II). The spectrum shows the only difference with Ia in the appearance of olefinic proton at 3.42τ (s, 1H) and decrease of the intensity of OH proton (1H, br.).

Although the degradative study has not been carried out due to the scarcity of the sample, disposition of the substituents on benzoquinone nucleus was suggested by the following evidences. As was discussed in the previous paper[1] and will be shown in this report, Ia and Ib are assumed to be sesquiterpenoids. Thus the methyl group and the trimethylcyclopentyl group should be in 1,4-positions. Recent revision[17] of the structure of perezone, a sesquiterpenoid benzoquinone from *Perezia* spp., from VIa to VIb clearly demonstrated that the both isomers are distinguishable by the absence and presence of allylic coupling of the methyl group with the adjacent olefinic proton

Table II. Nuclear Magnetic Resonance Spectra of Deoxyhelicobasidin and Related Compounds

	Quinonoid methyl (3H)	Quinonoid proton (1H)	Hydroxyl	Aliphatic methyl	Aliphatic methylene
Deoxyhelicobasidin (Ib)	8.06(s)	3.42(s)	2.82(1H)	9.23(s, 3H) 8.87(s, 3H) 8.69(s, 3H)	8.1~8.6(6H)
Helicobasidin[1] (Ia)	8.07(s)	——	2.73(2H)	9.15(s, 3H) 8.90(s, 3H) 8.65(s, 3H)	8.1~8.6(6H)
Perezone*[7,17] (VIb)	7.94(d) (J=1.5 c.p.s.)	3.53(q)	2.65(1H)	——	——
Dihydroperezone*[7,17] (VIIb)	7.93(d) (J=1.5 c.p.s.)	3.48(q)	2.92(1H)	9.18(d, 6H) 8.80(d, 3H)	8.2~8.9(7H) 7.0 (1H)
Isomer of VIIb*[7,17] (VIIa)	8.05(s)	3.52(s)	2.87(1H)	9.15(d, 6H) 8.87(d, 3H)	8.2~8.9(7H) 7.1 (1H)

All spectra are taken in $CDCl_3$ at 60 Mc. and expressed in τ values.

(Table II).*[7] Since the olefinic methyl group in Ib appears in a sharp singlet at 8.06τ, the hydroxyl group in Ib must be in the adjacent position. Slight shifts of the three

*[7] The authors' thanks are due to Professor Thomson and Dr. Archer, University of Aberdeen, for their kind donation of the copies of NMR spectra of the related compounds.

17) *a*) D.A. Archer, R.H. Thomson : Chem. Comm., **1965**, 354. *b*) E.R. Wagner, R.D. Moss, R.M. Brooker, J.P. Heeschen, W.J. Potts, M.L. Dilling : Tetrahedron Letters, **1965**, 4233. *c*) R.B. Bates, S.K. Paknikar, V.P. Thalacker : Chem. & Ind., **1965**, 1793. *d*) F. Walls, J. Padilla, P. Joseph-Nathan, F. Giral, J. Romo : Tetrahedron Letters, **1965**, 1577; F. Walls, M. Salmón, J. Padilla, P. Joseph-Nathan, J. Romo : Bol. Inst. Quim. Univ. nac. autón. México, **17**, 16 (1965); E. Cortés, M. Salmón, F. Walls : *Ibid.*, **17**, 19 (1965); J. Romo, *et al.* : Tetrahedron, **22**, 2387 (1966).

tertially methyl groups in Ib from those in Ia will also be explainable by the absence and presence of a hydroxyl group in the adjacent position.

Ib shows nearly the same $[\alpha]_D$ with Ia. Thus the compound (Ib), now designated deoxyhelicobasidin, should be expressed by the formula Ib. Quite recently existence of hydroxyperezone (VIc) in nature has been reported.[18] Thus helicobasidin (Ia) corresponds to the cyclic isomer of VIc, while deoxyhelicobasidin (Ib), to an isomer of hydroxyl group of the corresponding compound of perezone (VIb).

In the previous paper,[1] the biogenesis of helicobaidin (Ia) via sesquiterpene hydrocarbons, cuprenenes (VIII)[19] and cuparene (IX),[19~21] has been suggested. The biogenesis of the sesquiterpene hydrocarbons has been well elucidated by the relation with γ-bisabolene (X)[20~22] or with widdrol (XI).[23] Irrespective of the intermediate, the labelling pattern in Ia from radioactive mevalonate will be the same, if Ia is actually a sesquiterpene (Chart 2).

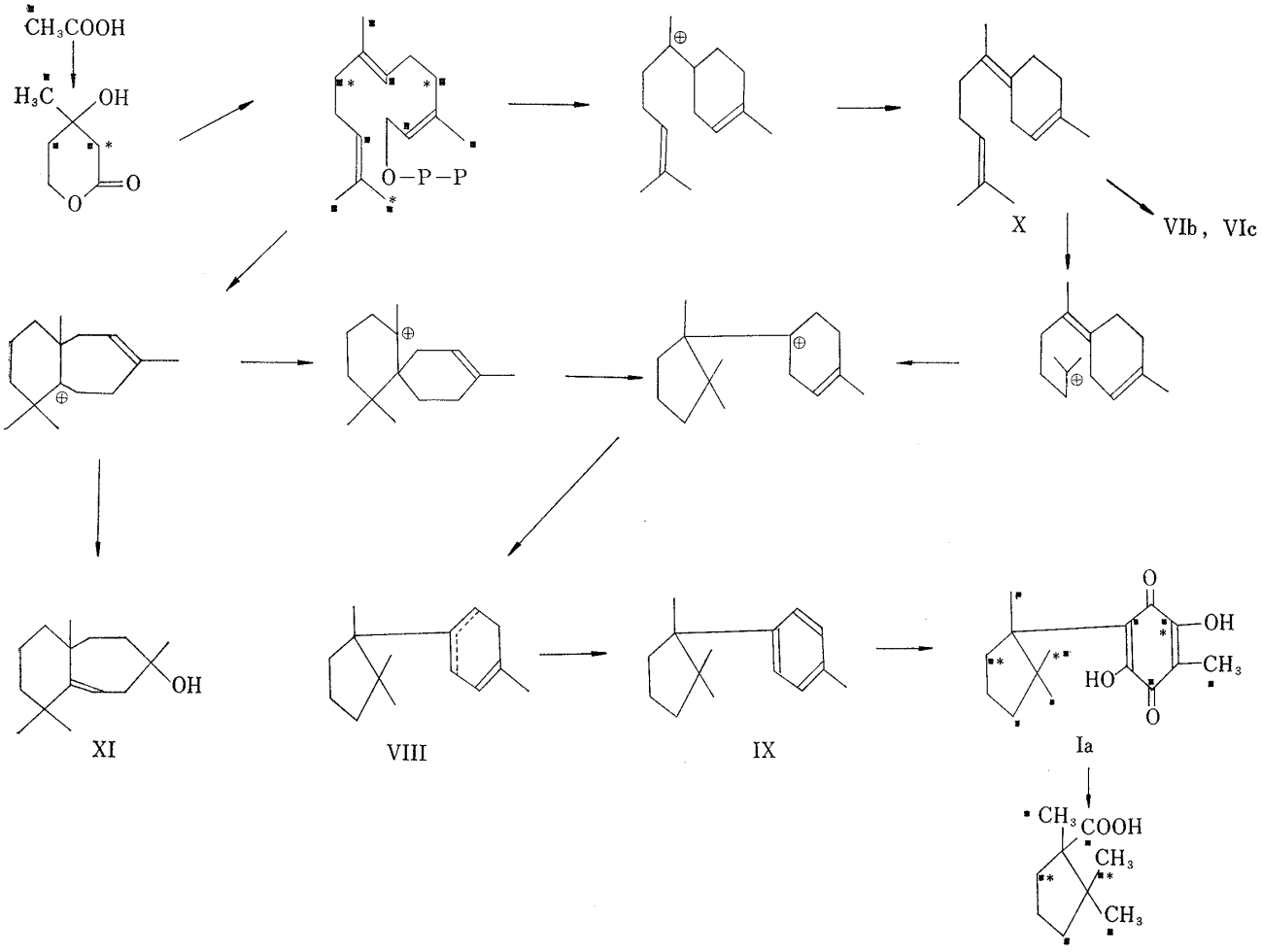


Chart 2. Biosynthesis of Helicobasidin

As for the phenolics of higher plants, biosynthesized entirely from mevalonate units, many compounds, such as thymol, thymoquinone, perezone,[17] hydroxyperezone,[18]

18) T. García, E. Domínguez, J. Romo : *Ibid.* **17**, 16 (1965).
19) T. Nozoe, H. Takeshita : Tetrahedron Letters, No. 23, 14 (1960).
20) C. Enzel, H. Erdtman : Tetrahedron, **4**, 361 (1958).
21) W. Parker, R. Ramage, R. A. Raphael : J. Chem. Soc., **1962**, 1558.
22) J. B. Hendrickson : Tetrahedron, **7**, 82 (1959).
23) W. G. Dauben, P. Oberhänsli : J. Org. Chem., **31**, 315 (1966).

totarol, quinones from *Mansonia altissima*,[24] fuerstione,[25] royleanones, and gossypol, have been known. In these mevalonate-originated phenolics, biosyntheses of thymol in *Orthodon japonicum*[26] and gossypol in *Gossypium herbaceum*[27] have been confirmed by the use of [^{14}C]-mevalonate.

Although the incorporation of mevalonic acid units into the side chains of some mold-producing phenolic substances, such as mycophenolic acid, auroglaucin and norherqueinone, have been confirmed by tracer experiments, Ia and Ib are, as far as the authors are aware, only the example of isoprenoid phenolics of mold metabolites.

The other metabolite of the mold, mompain (IIa), is assumed to be derived from acetate-malonate units. Acetate-malonate origin of mold naphthoquinones has recently been studied in javanicin in *Fusarium javanicum*,[28] mollisin in *Mollisia caesia*,[29] and elsinochrome A in *Elsinoë* spp.[30] Although the structure (IIa) is simple but is a rather unusal from biogenetical point of view, since IIa has no side chain such as methyl group, which shows the starting unit of the ring formation from acetate-polymalonates units.

From these points of view, biosynthesis of Ia and IIa in the mold was studied.

As the preliminary work, the growth of the mold and the formation of the pigments were followed in the course of incubation. As was shown in Fig. 3, the formation of Ia becomes rapidly after 60 days' incubation, following the logarithmic stage of the growth of the mold itself. On the contrary formation of IIa starts earlier than Ia, but the formation of intensively colored polymeric compounds makes the separation of the pigment difficult after a long period of cultivation. From these findings, the incubation time shown in Table III has been settled for tracer experiments. As the precursors of the biosynthetic study, [2-^{14}C]-acetate, [2-^{14}C]-malonate, and [2-^{14}C]-*dl*-mevalonic lactone were employed. Separation and purification of Ia and IIa have been carried out as shown in Experimental.

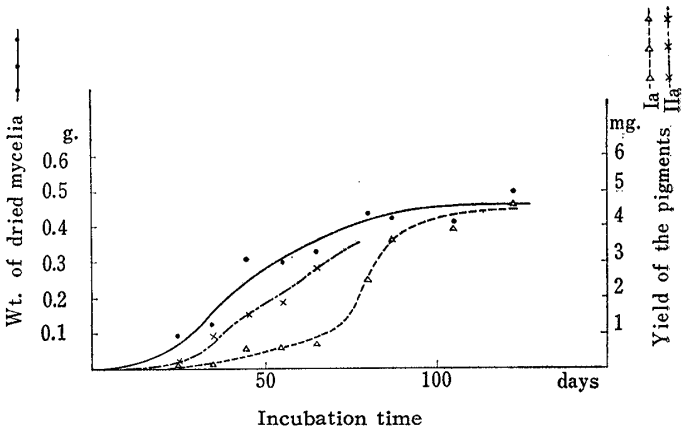


Fig. 3. Growth of the Mold and the Metaboslim of the Pigments (per 100 ml. medium)

The activity of the isolated pigments were determined by infinite thinness method and incorporation ratios are shown in Table III. Although incorporation of the three substrates was rather inferior, incorporation of mevalonate into Ia was found to be more than ten times of that into IIa and incorporation of acetate and malonate into IIa is much higher than that into Ia, the fact clearly indicating the different biosynthetic origin of the two metabolites.

Degradation after dilution of Ia has been carried out as follows : Alkaline hydrogen peroxide oxidation of Ia afforded *l*-camphonanic acid.[1] For comparison, Ia was derived to its leucoacetate[1] in order to carry out the determination of the radio-activity

24) G. B. M. Bettólo, C. G. Casinovi, C. Galeffi : Tetrahedron Letters, **1965**, 4857.
25) D. Karanatsios, J. S. Scarpa, C. H. Euguster : Helv. Chim. Acta, **49**, 1151 (1966).
26) M. Yamazaki, T. Usui, S. Shibata : This Bulletin, **11**, 363 (1963).
27) P. F. Heinstein, F. H. Smith, S. B. Tove : J. Biol. Chem., **237**, 2643 (1962).
28) S. Gatenbeck, R. Bentley : Biochem. J., **94**, 478 (1965).
29) R. Bentley, S. Gatenbeck : Biochem., **4**, 1150 (1965).
30) C.-T. Chen, K. Nakanishi, S. Natori : This Bulletin, **14**, 1434 (1966).

Table III. Incorporation of the Substrates into Helicobasidin and Mompain

Substrate	Amounts added (μc.)	Cultural medium (ml.)	Age of the addition of substrates (days)	Age of harvest (days)	Dry wt. of mycelia (g.)
Sodium [2-^{14}C]-acetate	100	150×5	35	43	1.94
Ethyl [2-^{14}C]-malonate	100	150×5	35	43	1.94
[2-^{14}C]-Mevalonic acid lactone	100	150×5	69	83	3.02

Substrate	Helicobasidin (Ia)			Mompain (IIa)		
	Total yield (mg.)	Specific activity (10^{-6}× d.p.m./mM)	Incorporation (%)	Total yield (mg.)	Specific activity (10^{-6}× d.p.m./mM)	Incorporation (%)
Sodium [2-^{14}C]-acetate	5.56	5.69	0.05	17.0	15.8	0.53
Ethyl [2-^{14}C]-malonate	11.2	3.63	0.07	6.93	8.60	0.12
[2-^{14}C]-Mevalonic acid lactone	30.2	2.19	0.11	25.8	0.11	>0.01

Table IV. Specific Activities of Helicobasidin and Mompain Derivatives

	Substrate					
	[2-^{14}C]-Acetate			[2-^{14}C]-Mevalonate		
	Specific activity (d.p.m./mM)	%	Theoretical (%)	Specific activity (d.p.m./mM)	%	Theoretical (%)
Helicobasidin						
Helicobasidin leucoacetate	5.80×10^5	100	100	2.01×10^5	100	100
l-Camphonanic acid	3.97×10^5	68.4	66.7(60)[a]	1.35×10^5	67.2	66.7(60)[a]
	3.74×10^5	64.5		1.37×10^5	68.2	

	Substrate					
	[2-^{14}C]-Acetate			[2-^{14}C]-Malonate		
	Specific activity (d.p.m./mM)	%	Theoretical (%)	Specific activity (d.p.m./mM)	%	Theoretical (%)
Mompain						
Mompain leucoacetate	2.91×10^5	100	100	1.39×10^5	100	100
p-Bromophenacyl ester of acetic adid formed from dimethylmompain (XII)	2.70×10^4	9.3	20(10)[a]	1.24×10^4	8.9	20(10)[a]
				1.24×10^4	8.9	

a) The value calculated from uniformly labelling of the carbon atoms.

using a liquid-scintillation counter. The results were shown in Table IV. *l*-Camphonanic acid, having nine carbon atoms out of fifteen of Ia, should show 6/9 and 2/3 respectively of the activity of Ia, when [2-^{14}C]-acetate and [2-^{14}C]-mevalonate were administered. As was shown in the Table, the observed values show good agreement with the theoretical value. The fact, along with the incorporation ratio, demonstrates the sesquiterpene nature of Ia (Chart 2).

After the examination of several methods for the degradation of IIa, C-methylation by Fieser's method,[31] followed by the Kuhn-Roth oxidation was employed. The

31) L. F. Fieser, *et al.*: J. Am. Chem. Soc., **70**, 73, 3179 (1948); J. R. Slangle, H. J. Shine: J. Org. Chem., **24**, 107 (1959).

structure of C-methylation product (3,6-dimethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone) (XII), m.p. >300°, was confirmed by NMR (δ 2.00 (6H) in $(CD_3)_2SO$, no methoxyl and ring proton). Acetic acid formed by the oxidation of XII was derived to its *p*-bromophenacyl ester and counted. However it showed about 10% of the activity of the leucoacetate of IIa and the value corresponds just half of the theoretical value calculated from acetate-polymalonate origin of IIa and agrees rather well with that calculated from non-specific labelling of IIa. At present we can not give any confirmative explanations of this result. It might be possible that the randamisation has occurred in the course of rather long period of incubation or IIa has been formed via an unexpected pathway.*8

Experimental*9

Structure of Mompain

Mompain (IIa)——Crude sample of mompain[2] was further purified by recrystallisation from water or dioxane to dark red leaflets, subliming around 200° and decomposing gradually at 300~360°. IIa is insoluble in petroleum, $CHCl_3$, and benzene and is soluble in alcohols. Thin-layer chromatography on Silicagel G treated with oxalic acid gave a single spot. The alkaline solution decolorised with $Na_2S_2O_4$ and also by catalytic hydrogenation. The $FeCl_3$ reaction gave a dark green coloration, $Mg(OAc)_2$ violet-red and $Pb(OAc)_2$ blue-violet precipitates. The aqueous solution showed orange color in acidic, red in neutral and violet in alkaline. IR cm^{-1}: 3240 (OH), 1660, 1625, 1602, 1582 (C=O, C=C), 1320, 1220, 1187, 1087, 870, 807. *Anal.* Calcd. for $C_{10}H_6O_6$: C, 54.05; H, 2.72; M.W., 222. Found: C, 54.18, 53.91; H, 2.73, 2.82; M.W. (Mass Spectrum), 222.

Mompain Tetraacetate (IIb)——IIa was acetylated with Ac_2O and H_2SO_4 by the ordinary method. Yellow needles from EtOH melted at 176~179°. IR cm^{-1}: 1778 (acetyl C=O), 1672 (sh), 1663, 1650 (sh), 1592, 1185, 1148, 1011, 922, 865. *Anal.* Calcd. for $C_{18}H_{14}O_{10}$: C, 55.37; H, 3.62. Found: C, 54.98; H, 3.48.

Mompain Dimethyl Ether (IIc)——i) IIa (30 mg.) in ether was methylated with ethereal diazomethane. Dark red precipitates separated immediately and recrystallised from dioxane to dark red needles (25 mg.) of m.p. 260~262°. IR cm^{-1}: 1627, 1604, 1574, 1284, 1230, 1095, 1002, 862, 807. *Anal.* Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.03. Found: C, 57.54; H, 4.49.

ii) IIa (15 mg.) was heated under reflux with methanolic-HCl (20%, 1 ml.) for 8 hrs. The reaction mixture was poured on a column of $CaHPO_4$ and the elute, freed from IIa, was evaporated and purified as before to give IIc (5 mg.).

Mompain Dimethyl Ether Diacetate (IId)——IIc was acetylated with Ac_2O and H_2SO_4. Recrystallization from acetone afforded orange-yellow needles, m.p. 249~250°. IR cm^{-1}: 1764, 1681, 1642, 1620, 1594, 1358, 1290, 1258, 1190, 1088, 1021, 910, 888. *Anal.* Calcd. for $C_{14}H_{14}O_8$: C, 57.49; H, 4.22. Found: C, 57.77; H, 4.38.

Mompain Tetramethyl Ether (IIe)——i) IIa (5 mg.), dimethyl sulfate (0.2 ml.) and K_2CO_3 (0.3 g.) in acetone (1 ml.) were refluxed for 1 hr. Deep red solid was separated by filtration, washed with acetone, dissolved in water, and, after acidification, extracted with ether. Removal of the solvent gave IIc (3 mg.). The combined acetone filtrate and washing were concentrated and passed through a column of alumina (neutral, grade III). A yellow band was collected and recrystallized from hexane-benzene to give yellow needles (2 mg.), m.p. 169~171°. IR cm^{-1}; 1674, 1644, 1628, 1546, 1357, 1260, 1237, 1220, 1098, 1039, 847.

ii) IIc (0.2 g.) was refluxed with Ag_2O (0.8 g.), CH_3I (0.6 ml.), and $CHCl_3$ (10 ml.) for 15 hrs. After filtration and evaporation, the residue was treated as before to obtain IIe (30 mg.). *Anal.* Calcd. for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found: C, 60.64; H, 4.93.

Mompain Leucoacetate (III)——IIa (0.1 g.) was refluxed with a mixture of Ac_2O (5 ml.), AcOH (1 ml.), NaOAc (0.3 g.), and Zn dust (0.6 g.) for 1 hr. Recrystallization from acetone-water afforded colorless prisms of m.p. 229~232°. *Anal.* Calcd. for $C_{22}H_{20}O_{12}$: C, 55.44; H, 4.23. Found: C, 55.70, 55.30; H, 4.51, 4.57.

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*8 (Note Added in Proof): Quite recently shikimic acid origin of 2-hydroxynaphthoquinone in *Impatiens balsamina* and of V. K_2 in *E. coli* has been demonstrated (D. Chen, B. A. Bohm: Canad. J. Biochem., **44**, 1389 (1966); G. B. Cox, F. Gibson: Biochem. J., **100**, 1 (1966)).

*9 Unless otherwise specified, UV spectra were determined in EtOH solution on a Hitachi EPS-2 recording spectrophotometer and infrared spectra were taken on Koken Model 301 infrared spectrophotometer in Nujol mull.

Desacetylspinochrome A (M) Dimethyl Ether (Mompain Dimethyl Ether) (IIc)[*6]——A mixture of spinochrome A (M) (IV) (25 mg.) and conc. H_2SO_4 (0.5 ml.) was heated at 100° for 2 hrs. The mixture was poured into an ice-water, the precipitates were extracted with ether and dried. The thin-layer chromatography of the solution showed one clear spot having the same Rf value with mompain (IIa), in addition to a weak spot of the starting material. The solution was added to an ethereal solution of diazomethane, ether was removed, and the residual solid was passed through a column of $CaHPO_4$. The red band was eluted with a mixture of benzene-ethyl acetate (4:1) and the recrystallized from dioxane to dark orange needles (2 mg.), m.p. 266~268°, which showed the identity with mompain dimethyl ether (IIc) by a mixed fusion, IR spectra, and thin-layer chromotography in two solvent systems.

Structure of Deoxyhelicobasidin

Deoxyhelicobasidin (Ib)——Crude sample of helicobasidin[1,2] was passed through a column of acid-washed silica-gel (Mallinckrodt). Three bands appeared by the elution with hexane-benzene (9:1). After the elution of first band, which is predominant in amount and composed with helicobasidin (Ia), the second band was collected and purified further by crystallization from hexane to yellow needles of m.p. 194~195°, $[\alpha]_D^{25}$ −186.5° (c, 0.375, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.51; H, 8.26. UV λ_{max}^{EtOH} mμ (log ε): 274, 404 (4.11, 3.02). IR cm^{-1}: 3275 (OH), 1664, 1633 (s), 1619 (sh), 1594 (C=O, C=C), 1382 (s), 1354, 1290 (s), 1155, 1143. NMR (Table II).

Biosynthesis of Helicobasidin (Ia) and Mompain (IIa)

Cultivation——The organism used in this study was *Helicobasidium mompa* TANAKA, supplied by Dr. S. Takai, Government Forest Experiment Station, Tokyo.[*10] It was maintained on slopes of malt agar. For inoculation, a piece of the mycelium and agar was transferred to a medium of the following composition: KH_2PO_4, 0.5 g.; K_2HPO_4, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.2 g.; $MnSO_4 \cdot 7H_2O$, 0.01 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; NaCl, 0.01 g.; sucrose, 20.0 g.; L-asparagine, 3.0 g.; vitamins, 1/3 tablet of "Neopanto"-zyo; water to 1L.; pH, ca. 6.0. For the preliminary work without the addition of isotopes, 200 ml. portions of the medium were used in Roux flasks. In experiments with isotopically labelled substrates, 150 ml. portions of the medium were used.

Incubation has been carried out stationally at 25°.

Separation and Determination of Ia and IIa——In the preliminary work, 5~10 flasks were taken out for some intervals in the course of cultivation. After filtration of the cultural medium, the mycelium pads were washed with a little water. The dried mycelia were extracted with hexane, concentrated, and the residue was applied to preparative thin-layer chromatography, using the plates of Silicagel G treated with oxalic acid solution and the developing solvent of a mixture of hexane and benzene (4:6). The main band was collected and extracted with EtOH and the solution was adjusted to a definite volume to determine the optical density at 296 mμ.

The culture filtrate and the washing were made acidic with conc. HCl, extracted with ether, and evaporated. The residue was extracted with hot water and with 10% EtOH and the combined extracts, after acidification, was extracted with ether. The ethereal extract was concentrated and passed through a column of acid-washed silicic acid (Mallinckrodt) and developed with a mixture of benzene-EtOAc-HCOOH (95:5:1). The main band was collected, evaporated and dissolved in EtOH and the optical density at 555 mμ was determined.

Growth of the mycelia and yields of Ia and IIa were shown in Fig. 3.

Preparation and Separation of Radioactive Ia and IIa——The substrates in a minimum amount of distilled water were added below the mycelium pad after preincubation for a period of time shown in Table III. After further incubation as shown in the table, the culture was treated with the same procedure as mentioned before, except the separation of Ia being carried out by a column chromatography through acid-treated silicic acid. The ethanolic solutions of Ia and IIa thus obtained were used for the determination of the total amounts by their ultraviolet absorptions and of the radioactivity by infinite thinness method using an Aloka-type windowless Q-gas flow counter. The results were shown in Table III.

Then the samples were diluted with the carriers and recrystallized from the solvents shown before.[1]

[^{14}C]-Helicobasidin Leucoacetate——Prepared by the method reported before.[1] M.p. 156~159° from hexane-EtOAc.

Alkaline Hydrogen Peroxide Oxidation of Ia——*l*-Camphonanic acid was obtained and purified by the reported method.[1] M.p. 185~188°.

[^{14}C]-Mompain Leucoacetate——Prepared by the method shown in this paper. M.p. 235~238° from hexane-acetone.

Dimethylmompain (3,6-Dimethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone) (XII)——To AcOH solution (80 ml.) of IIa (300 mg.), maintained at 85~90°, excess of Ac_2O_2 in ether (25 ml.) was added gradually for 5 hrs. according to the method of Slangle and Shine,[31] and, after further heating for 1 hr., HOAc was removed under a reduced pressure. The residue was passed through a column of acid-washed silicic acid

[*10] cf. S. Takai: Phytopathol. Z., **43**, 175 (1961/62).

and eluted with a mixture of benzene–EtOAc–HCOOH (95:5:1). The main band was collected and recrystallized from benzene to dark violet needles of XII (32 mg.), m.p. >300° (decomp.) after darkening around 260°. NMR ($(CD_3)_2SO$) : δ 2.00 (6H), 13.6. *Anal.* Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.03. Found : C, 58.09; H, 4.22.

The radioactive sample was prepared by the same method.

The reaction condition was followed by the use of thin-layer chromatography and we have learned that, if the reaction mixture has been heated for a short period of time, it shows the presence of other reaction product, which has been isolated by the chromatography and proved to be monomethyl compound, deep violet needles from benzene, m.p. >300° (decomp.). NMR ($(CD_3)_2SO$) : δ 1.94 (3H), 6.60 (1H), 13.2.

The Kuhn-Roth Oxidation of XII——The oxidation has been carried out by the method of Eisenbraun, *et al.*[32] Acetic acid was recovered by steam-distillation, the fraction being titrated with 0.02*N* NaOH. The NaOAc recovered on evaporation was derived to its *p*-bromophenacyl ester by the conventional method and purified by passing a column of silicic acid. Recrystallization from hexane or EtOH gave colorless needles of m.p. 85~87°.

Determination of the Radioactivities——The samples (2~10 mg.) were accurately weighed out and dissolved in 2 ml. EtOH and 18 ml. of scintillator (prepared from PPO (7 g.), dimethyl-POPOP (0.3 g.), and dioxane-naphthalene (100 g.)). The activities were determited by channel-ratio method using a Packard Tricarb liquid scintillation counter.

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32) E. J. Eisenbraun, S. M. McElvain, B. F. Aycock : J. Am. Chem. Soc., **76**, 607 (1954).