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Perspective

Compactin (ML-236B) and Related Compounds as Potential Cholesterol-Lowering Agents That Inhibit HMG-CoA Reductase

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One of the major causes of death in the U.S. and other developed countries is coronary heart disease. Approximately 800 000 Americans die of it a year, amounting to 40% of all deaths. Coronary heart disease actually is a wide assortment of diseases. The basic manifestation of many of them is atherosclerosis, caused when fatty deposits called plaque build up on the inner walls of arteries. Cholesterol is a major component of the atherosclerotic plaque. Many scientists believe that a high level of cholesterol in the blood is a major contributor to the development of atherosclerosis. Since in humans the greater part of the cholesterol in the body is synthesized de novo, mostly in the liver, the search for drugs to inhibit cholesterol biosynthesis has long been pursured as a means to lower the level of plasma cholesterol and so help to prevent and treat atherosclerosis.

Cholesterol is synthesized from acetyl-CoA via a series of more than 20 enzymatic reactions. This synthetic pathway is mainly regulated by the activity of the enzyme 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate. In many tissues, changes in the activity of this enzyme are closely related to changes in the overall rate of cholesterol synthesis over a wide range of physiological conditions. This enzyme is, therefore, a prime target for pharmacological intervention.

Endo and his associates at Sankyo Co. (Tokyo), who had tested 8000 strains of microorganisms for their ability to produce an inhibitor of sterol synthesis in vitro, first discovered three active compounds, designated ML-236A, ML-236B, and ML-236C, in the culture broth of the fungus *Penicillium citrinum*.[1] The main compound ML-236B (generic name: mevastatin) is identical to one later isolated from *Penicillium brevicompactum* as an antifungal agent named compactin.[2] ML-236B has been shown to be a specific inhibitor of HMG-CoA reductase and highly effective in lowering plasma cholesterol levels in animals and men.

Isolation and Chemistry of Compactin (ML-236B) Related Compounds. Another active compound related to compactin (named monacolin K) was isolated from the fungus *Monascus ruber* by Endo.[3,4] The same compound (named mevinolin) was also isolated by Alberts et al. from *Aspergillus terreus*.[5] In addition to these products, several related metabolites were isolated from cultures of these fungi, which include dihydrocompactin from *P. citrinum*,[6] dihydromevinolin from *A. terreus*,[7] monacolin J and L from *M. ruber*,[8] and dihydromonacolin L and monacolin X from a mutant strain of *M. ruber*.[9] All these metabolites are structurally related to each other (Figure 1) and are specific inhibitors of HMG-CoA reductase.

Several active compounds have also been derived from either compactin or monacolin K by microbial conversion (Figure 2). 3β-Hydroxycompactin, 6α-hydroxyisocompactin and 3-hydroxymonacolin K are produced by growing the fungus *Mucos hiemalis* in a culture medium that contains, in addition to nutrients, compactin and monacolin K, respectively.[10] Compactin is also converted to 3α-hydroxycompactin by *Syncephalastrum nigricans*[11] and to 6α-hydroxyisocompatin by *Absidia coerulea*.[12] 8a-Hydroxycompactin and 8a-hydroxymonacolin K are derived from compactin and monacolin K, respectively by growing *Schizophyllum commune*.[13] Phosphorylated

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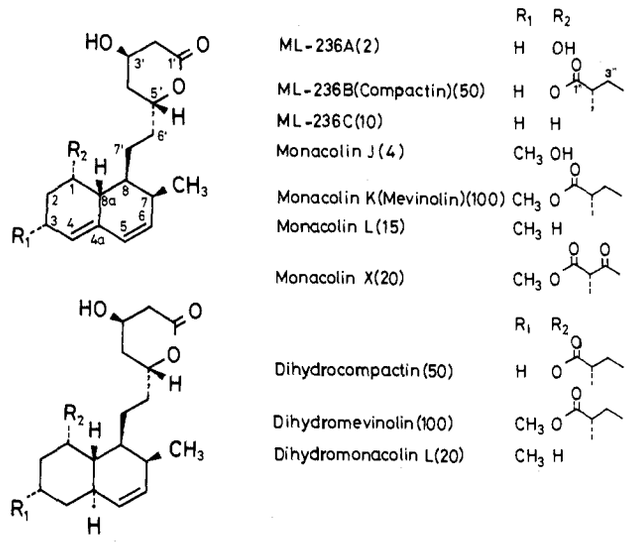

Figure 1. Compactin (ML-236B) related compounds of microbial origin. Numbers in the parentheses represent relative activity to inhibit rat liver HMG-CoA reductase.

derivatives (5′-phosphocompactin acid and 5′-phosphomonacolin K acid) are produced by several fungal strains.[14]

Recently new microorganisms producing compactin have been isolated by Endo et al. (unpublished data), which include *Paecilomyces* sp. and *Hypomyces chrysospermus*. These fungal strains are particularly suited for the production of compactin, since no mycotoxin has been isolated from culture broth of these fungi. Other producers, *Penicillium citrinum* and *P. brevicompactum*, are known to produce mycotoxins.[15] Compactin-related compounds are produced as the water-soluble acid form which is converted to the water-insoluble lactone form by acidification or drying except for the 5′-phosphorylated derivatives (Figure 3).

Mechanism for HMG-CoA Reductase Inhibition. The inhibition of HMG-CoA reductase by compactin and related compounds is reversible.[16,17] As can be expected from the structure of their acid forms, the inhibition by these compounds is competitive with respect to HMG-CoA. The K_i value for the acid form of compactin, which is determined from the partially purified rat liver enzyme, is $\sim 1 \times 10^{-9}$ M, while under the same conditions, the K_m value for HMG-CoA is $\sim 10^{-5}$ M.[17] Thus, the affinity of HMG-CoA reductase for compactin is 10 000-fold higher than its affinity for the natural substrate HMG-CoA, showing compactin to be a highly potent inhibitor.

Compactin does not affect other enzymes involved in cholesterol biosynthesis.[18] In addition, almost all studies on compactin with cultured cells and intact animals suggest that reductase is the only enzyme that is inhibited by compactin (see below).

Structure–Activity Relationships at Enzyme Level. Structural similarity between HMG-CoA and compactin-related compounds suggests that the active center of these agents in the inhibition of HMG-CoA reductase is at the δ-lactone moiety of the molecules. This hypothesis is supported by the data that inhibitory activity of compactin is reduced to $^1/_{100}$ or less by acetylation of the hydroxy group at either $C_{3'}$ or $C_{5'}$ (unpublished data) and that 5′-phosphocompactin acid and 5′-phosphomonacolin K acid are $^1/_{10}$ and $^1/_{20}$ of compactin and monacolin K in the inhibitory activity, respectively.[14]

Other portions of compactin molecule also seem to be involved in inhibitory activity (Figure 1). Among them, the α-methylbutyrate ester plays a significant role, since analogues that lack such an ester (ML-236A and monacolin J) are $^1/_{25}$ in the activity, as compared with their respective counterparts (compactin and monacolin K).

The decalin ring of compactin-related compounds is essential to the inhibitory activity. This is shown by the data that HMG is more than 10^6-fold less active than compactin.[19] Dihydrocompactin, dihydromevinolin, and dihydromonacolin L are comparable in the activity to compactin, monacolin K, and monacolin L, respectively.[6,7,9]

Monacolin K analogues that have a methyl group at C_3 are twice as active as their respective compactin analogues (Figure 1), indicating a contribution of the methyl radical to potency. However, hydroxylation at C_{8a}, C_3, or C_6 gives no significant effect.[10-13]

Inhibition of Sterol Synthesis in Cultured Cells and in Animals. Compactin significantly inhibits cholesterol biosynthesis in a variety of cultured animal and human cells at as low as nM (10^{-9} M) concentration.[20,21] In cultured human skin fibroblasts, inhibition of sterol synthesis from [^{14}C]acetate is 50% at 1 nM, 80% at 10 nM, 90% at 100 nM, 95% at 1 μM, and 100% at 10 μM, respectively.[20] Under these conditions, sterol synthesis from [^{14}C]mevalonate and fatty acid synthesis from [^{14}C]acetate are not significantly affected.

The inhibition of HMG-CoA reductase by compactin results in the reduction of mevalonate production. As shown in Figure 4, in addition to cholesterol, ubquinones and dolichols which are involved in electron transport in the mitochondria and glycoprotein synthesis are also derived from mevalonate. In human skin fibroblasts which are grown in the presence of LDL (low-density lipoprotein)–cholesterol, compactin has no detectable effects on the synthesis of these two isoprenoids at 10 nM, a concentration that causes over 50% inhibition of sterol synthesis (unpublished data). When HMG-CoA reductase is partially suppressed by compactin, cells must have some way of diverting the small amounts of synthesized mevalonate preferentially into these crucial nonsterol products.

At higher concentrations where sterol synthesis is reduced by over 90%, compactin inhibits cell growth.[20] This inhibition can be overcome and cells can grow normally if a small amount of mevalonate is added to the culture medium. The compactin inhibition of growth and its reversal by mevalonate have been reported in other cultured cells and in animals.[22-30] These findings strongly suggest

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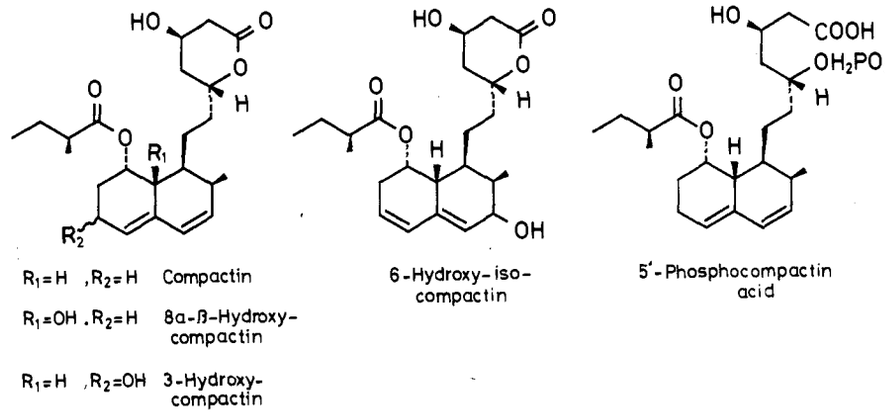

Figure 2. Compactin (ML-236B) related compounds produced by microbial conversion.

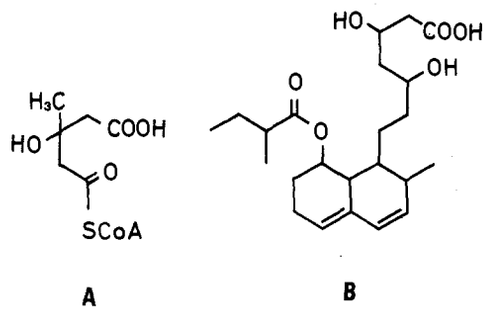

Figure 3. HMG-CoA (A) and the acid form of compactin (B).

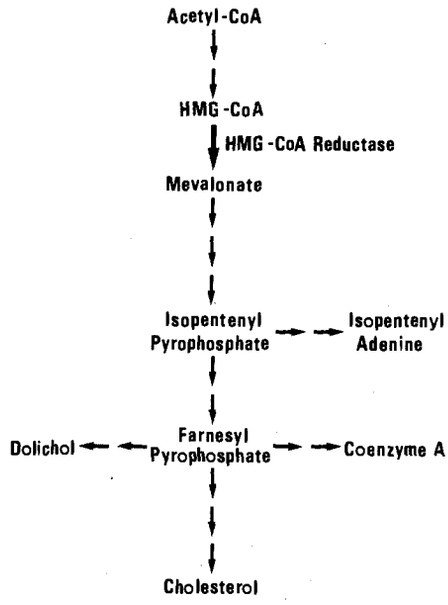

Figure 4. Synthetic pathway for cholesterol, ubiquinones, and dolichols. Compactin inhibits conversion of HMG-CoA to mevalonate catalyzed by HMG-CoA reductase.

that compactin is a specific inhibitor of HMG-CoA reductase.

Hypocholesterolemic Activity. When given orally, compactin is effective in lowering plasma cholesterol levels in man and some animal species, notably the chicken,[21] rabbit,[31] dog,[32] and monkey.[33] In other species, such as the rat,[34] mouse,[34] and hamster,[21] the feeding of compactin does not decrease plasma cholesterol.

In the rat, the inhibition of hepatic cholesterol synthesis by compactin administration is accompanied by a large (5- to 10-fold) increase in HMG-CoA reductase activity in the liver.[34] In addition, compactin causes in the rat a significant decrease in the fecal elimination of bile acids which are synthesized from cholesterol in the liver.

In the dog and monkey,[32,33] compactin produces a rapid reduction of plasma cholesterol levels at a daily dose of 10–20 mg/kg. Plasma triglyceride is not consistently lowered. Of the plasma lipoproteins, LDL, which are known to be atherogenic, are reduced, but HDL (high-density lipoproteins), antiatherogenic lipoproteins, are not affected. Compactin stimulates hepatic HMG-CoA reductase in the dog, but the increase is of considerably lesser magnitude than in the rat (unpublished data). In addition, fecal excretion of bile acids is, also unlike in the rat, not reduced but rather slightly elevated by compactin both in the dog and monkey.[32,33] These data suggest that the lack of hypocholesterolemic effect in the rat is due, at least partly, to the large increase in hepatic HMG-CoA reductase activity and to the decrease in the bile acid excretion. Metabolic disposition of compactin in the rat has not yet been reported.

In normal rabbits, compactin and mevinolin reduce significantly plasma cholesterol levels at a daily dose of 5 and 2 mg/kg, respectively.[31,35] Essentially the same activity is obtained in rabbits with inborn hyperlipidemia (called WHHL rabbits).[33]

In healthy persons, compactin and mevinolin are well tolerated and exert a rapid and profound cholesterol-lowering effect at a dose of 5–10 mg/day.[36,37] Of the various types of hypercholesterolemia, FH (familial hypercholesterolemia) characterized by a marked increase in plasma LDL, are notably resistant to drug therapy. Hyperlipidemia is present in such persons and cardiovascular disease often, but not always, occurs prematurely. In

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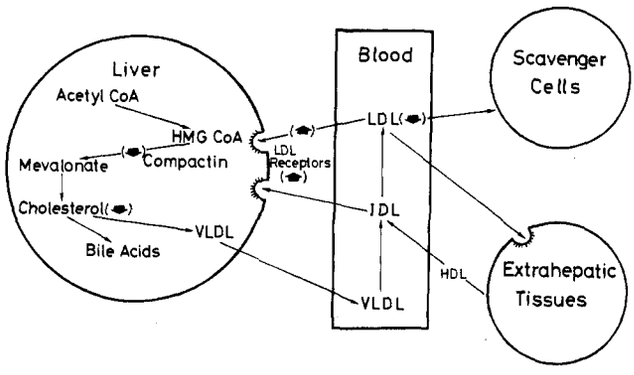

Figure 5. Biosynthesis and metabolism for cholesterol and lipoproteins and possible mechanisms for action of compactin. Arrows in the parentheses represent increase (↑) or decrease (↓) by compactin.

patients with heterozygous FH, Yamamoto et al. obtained a 27% decrease in plasma cholesterol after 4–8 weeks of compactin treatment at a dose of 60–100 mg/day.[38] Plasma cholesterol started to decrease within 2 weeks of treatment. According to Mabuchi et al.,[39] a 29% decrease in plasma cholesterol is obtained in heterozygous FH patients treated with compactin for 24 weeks at a dose of 30–60 mg/day. The reduced cholesterol levels is sustained during the period of drug treatment and returns to the pretreatment concentrations after terminating drug therapy. LDL are the only lipoprotein fractions that are decreased by compactin treatment; HDL are slightly elevated. Plasma triglyceride levels are not significantly lowered.

A much greater decrease in plasma LDL levels can be obtained by the combination of compactin and cholestyramine, bile acid binding resin, than can be produced by either agent alone.[40,41] While the reduction of LDL-associated cholesterol in patients with heterozygous FH is 28% by cholestyramine (12 g daily), that obtained by the combination of cholestyramine (12 g daily) and compactin (30 mg daily) is as high as 53%.[40] This combination seems to be the most effective therapy of hypercholesterolemia so far reported. The combination effect of these two drugs could be due to the inhibition by compactin of the increased cholesterol synthesis caused by cholestyramine treatment, since cholestyramine is known to induce hepatic HMG-CoA reductase activity in animals.[34] Some FH patients in Japan have been on the compactin treatment for over 2 years with no serious side effects. Compactin is much more effective in patients with hypercholesterolemia other than FH; a significant cholesterol-lowering activity is obtained at a dose of 5–10 mg/day.[36,42]

Compactin Effects in VLDL and LDL Metabolism. The greater part of the daily supply of body cholesterol is endogenously provided by synthesis, mainly in the liver, where the endogenous cholesterol is used in VLDL synthesis as a structural component. VLDL enter the circulation and are responsible for the transport and delivery of cholesterol as well as triglyceride to extrahepatic tissues (Figure 5).[43,44] In the circulation, VLDL becomes a substrate for lipoprotein lipase. As lipolysis occurs, VLDL become smaller and form remnants called IDL (intermediate-density lipoproteins) (Figure 5). In man, IDL serve as the precursors of LDL, which are the major carrier of cholesterol to extrahepatic tissues.

LDL in the circulation are taken up by extrahepatic cells by a LDL receptor-mediated process.[43] This process involves the binding of LDL to specific, high-affinity binding sites located on the surface of plasma membranes. Once binding has occurred, the LDL–receptor complex is internalized by endocytosis and digested by lysosomal enzymes to liberate free cholesterol. This cholesterol is utilized as an important structural component for cell membranes and in several tissues as the precursor for the synthesis of steroid hormones.

An additional pathway for LDL metabolism involves a lower affinity process associated with scavenger cells or macrophages of the reticuloendothelial system (Figure 5). In man, one-third to two-thirds of the plasma LDL is cleared by the high-affinity receptor-mediated process, and the remainder is degraded by the scavenger cells.

Although HDL are initially formed in the liver and intestine, they become modified in the circulation by interaction with the other lipoproteins and extrahepatic cells. It has been proposed that HDL are involved in removing cholesterol from extrahepatic cells. The HDL–cholesterol are then transferred to IDL via a plasma exchange protein.

As mentioned, compactin does lower plasma LDL–cholesterol levels both in animals and man but does not show a significant effect on HDL and IDL. There are two possible mechanisms for lowering plasma LDL levels by a HMG-CoA reductase inhibitor, namely, decreasing LDL synthesis and increasing its clearance from the circulation.

The inhibition of cholesterol synthesis in the liver by compactin is expected to decrease hepatic cholesterol content, which would be partly compensated by the decrease in VLDL synthesis and thus in its transport to the circulation. The reduced supply of plasma VLDL, in turn, would induce the decrease in IDL and then LDL synthesis. This hypothesis is supported by the data that mevinolin treatment of the dog produces a significant decrease in the synthetic rate for plasma LDL.[45] In FH heterozygotes studied by Bilheimer et al.,[46] however, the LDL synthesis does not change significantly by mevinolin treatment. The inhibition of VLDL and IDL synthesis by compactin or mevinolin and of VLDL supply to the circulation has not yet been shown and remains to be studied (Figure 5).

Secondly, the decreased content of hepatic cholesterol due to compactin inhibition is expected to be partly compensated by stimulating the hepatic uptake and degradation of plasma LDL. This hypothesis is supported by studies both in the dog and man.[45,46] In the dog, mevinolin raises the number of hepatic LDL receptors, which, in turn, enhances receptor-dependent uptake and degradation of plasma LDL (Figure 5). The receptor-independent clearance by scavenger cells does not change by mevinolin

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treatment. The receptor-dependent clearance of plasma LDL is also significantly increased in FH heterozygotes by mevinolin treatment.[46] Thus, LDL lowering in these patients can be explained by the increased LDL receptor activity.

Conclusion. In the Japanese population, as well as the European and American populations, FH occurs at a frequency of 1 in 500. It is closely associated with premature atherosclerotic heart disease. The increased levels of LDL in FH is believed to accelerate atherosclerosis.

FH is a disorder characterized by the defect in LDL receptor, which causes a decrease in LDL uptake by the cell. The decreased LDL uptake, in turn, results in higher levels of both plasma LDL and cellular HMG-CoA reductase activity. Thus, treatment should increase LDL catabolism by stimulating the LDL pathway. As mentioned, treatment with compactin (or mevinolin) is ideal in FH in that it reduces plasma LDL levels by stimulating the LDL receptor-mediated catabolism.

Of the analogues related to compactin, the acid form of 3β-hydroxycompactin (CS-514) (Figure 2), which is comparable to compactin in both in vitro and in vivo activities, has recently been reported to be superior to the parent compound in safety.[47] Compactin-related compounds have not been widely used in the treatment of hypercholesterolemia. Yet the studies with these agents have established a general principle: a competitive inhibitor of HMG-CoA reductase can reduce LDL levels in plasma by increasing LDL receptor without depleting vital body stores of cholesterol.

Registry No. HMG-CoA reductase, 9028-35-7; compactin, 73573-88-3; cholesterol, 57-88-5.

(47) S. Fujii, M. Arai, Y. Tsujita, and M. Tanaka, presented at the 16th General Meeting of the Japanese Society of Atherosclerosis (Tokyo), July 22–23, 1984.

Communications to the Editor

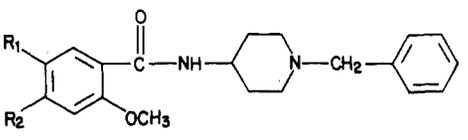
Novel Photoaffinity Label for the Dopamine D_2 Receptor: Synthesis of 4-Azido-5-iodo-2-methoxy-*N*-[1-(phenylmethyl)-4-piperidinyl]benzamide (Iodoazidoclebopride, IAC) and the Corresponding ^{125}I-Labeled Analogue (^{125}IAC)

Sir:

Dopamine agonist elecited behaviors (rotation, psychotomimetic actions, antiparkinsonian action, stereotypy, and locomotion) appear to be mediated by dopamine D_2 receptors in the brain.[1]

Although the dopamine D_2 receptor has been solubilized by several laboratories,[2] attempts to isolate and purify the protein by affinity chromatography[3] or photoaffinity labeling[4,5] have been relatively unsuccessful.[6,7] Alkylating type irreversible ligands have been developed (i.e., [^{3}H]-*N*-(chloroethyl)norapomorphine, phenoxybenzamine, *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline, flupenthixyl chloride), but have been too low in receptor affinity and/or selectivity to be of value in dopamine D_2 receptor isolation.[3,8-10] The molecular characterization of dopamine D_2 receptors has been hampered by the lack of specific photoaffinity probes which can be used to covalently label these sites.

We report here the synthesis of a series of substituted benzamides (Ib–Ig) and in particular Ig, an agent, useful as a photoaffinity label, which selectively and irreversibly labels dopamine D_2 receptors upon light irradiation.



Ia, R_1 = Cl; R_2 = NH_2; clebopride
Ib, R_1 = Cl; R_2 = N_3; azidoclebopride (AC)
Ic, R_1 = H; R_2 = NH_2;
Id, R_1 = I; R_2 = NH_2; iodoclebopride
Ie, R_1 = I; R_2 = N_3; iodoazidoclebopride (IAC)
If, R_1 = ^{125}I; R_2 = NH_2; [^{125}I]iodoclebopride
Ig, R_1 = ^{125}I; R_2 = N_3; [^{125}I]iodoazidoclebopride (^{125}IAC)

Since clebopride (Ia) was reported as a selective D_2 receptor antagonist,[11-13] we selected the substituted benzamide as a ligand that has inherent affinity for the binding site of D_2 receptors and incorporated an azido group as a photosensitive functional group, replacing the amino group in Ia. When Ib was photoactivated with light, it was capable of forming a covalent bond at or near the binding site.[14,15] The association with the recognition site will ordinarily be reversible until photolysis is initiated; the covalent linkage thus formed between the photoprobe and the binding site will thus facilitate the characterization and isolation of the dopamine D_2 receptor.

Clebopride (Ia) when reacted with sodium nitrite and concentrated hydrochloric acid formed the intermediate diazonium salt which was treated with an aqueous solution

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