Identification of Compounds Characterizing the Aroma of Fresh Whitefish (*Coregonus clupeaformis*)

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Volatile compounds collected on Tenax GC at room temperature (21 °C) from direct headspace purging of fresh uncooked whitefish (*Coregonus clupeaformis*) were separated by gas chromatography and identified by mass spectrometry, odor quality, and retention indices (I_E). Two distinct families of compounds previously associated with cucumber and melon fruits and mushrooms were identified as principal contributors to fresh whitefish aroma. Compounds responsible for a strong cucumber-like top note aroma were identified as (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienal, and 6-nonen-1-ol. 1-Octen-3-ol, 1-octen-3-one, 1,5-octadien-3-ol, 1,5-octadien-3-one, and 2,5-octadien-1-ol were principal contributors to a heavy, plant-like aroma. Twelve compounds were found above reported threshold concentrations and thus appear to contribute to the overall characterizing aroma of fresh whitefish.

Flavors and aromas commonly associated with fish and seafoods have been the subject of many investigations and reviews (Obata et al., 1950; Jones, 1961, 1967; Meijboom and Stroink, 1972; McGill et al., 1974, 1977; Swoboda and Peers, 1977; Aitken and Connell, 1979; Ikeda, 1980), but the definitive flavor chemistry of these systems is lacking. Ammonia, dimethylamine, and trimethylamine have long been implicated in fishy aromas and flavors (Dyer and Mounsey, 1945; Moncrieff, 1944; Stansby, 1962; Jones, 1967), but their role appears to be restricted to a modifying effect because it can be readily demonstrated that they do not provide the characterizing aroma to most seafoods. Reay and Shewan (1949) have claimed that trimethylamine contributes to stale fish aromas because fresh fish contains little of this compound. Other compounds have been implicated in fishy aromas and flavors (Forss et al., 1960; Badings, 1970, 1973; Meijboom and Stroink, 1972; Ke et al., 1975; Swoboda and Peers, 1977; Ikeda, 1980), but little agreement on influential compounds exists. However, it now appears that several distinctly different fish-like odors and flavors occur, and most of the conflicting data can be resolved by accepting a multiple fishy aroma concept.

Oxidized fish oils exhibit distinctly discernible fishy aromas that are provided by autoxidation of ω-3 fatty acids, and the off-flavor found in turkeys fed tuna oil characterizes this type of flavor (Crawford et al., 1975; Crawford and Kretch, 1976). It has been suggested (Badings, 1965, 1973) that the fishy odor may be attributed to the presence of a complex mixture of carbonyls. 2,4,7-Decatrienal (Badings, 1970; Meijboom and Stroink, 1972; Swoboda and Peers, 1977) and the 2,4-decadienals (Swoboda and Peers, 1977) together have been proposed as playing a dominant role in these oxidized fish oil-like flavors.

Other lipid oxidation products are well-known in seafood products (Yu et al., 1960; Aitken and Connell, 1979; Ikeda, 1980), but their role appears largely confined to a general contribution to oxidized flavors. However, (*Z*)-4-heptenal appears to characterize the cold-stored flavor of fish, especially frozen cod (McGill et al., 1974, 1977). It is produced from the oxidation of unsaturated fatty acids located primarily in the phospholipids (Ross and Love, 1979).

Flavors of cooked seafoods and fish undoubtedly reflect some of the compounds already mentioned, but the distinctive flavors of cooked fish appear to be characterized by reaction flavor compounds formed during cooking (Pokorny, 1980). The nature of these flavors has not been described chemically. However, dimethyl sulfide provides a characterizing top-note aroma to cooking or stewing oysters and clams (Yueh, 1961; Ronald and Thomson, 1964; Mendelsohn and Brooke, 1968) and arises principally through the thermal degradation of dimethyl-β-propriothetin (Motohiro, 1962). Fish house, boiled crab, and lobster aromas appear to constitute another group of related fishy aromas, but compounds which yield them remain obscure. Obata et al. (1950) reported that piperidine, δ-aminovaleric acid, and δ-aminovaleraldehyde had fishy aromas and that the reaction products of piperidine and piperidine aldehyde exhibited a freshwater fish aroma. When trimethylamine was added to this mixture, the aroma of saltwater fish was reportedly achieved. However, these results have never been confirmed.

All fresh uncooked fish are characterized by a common green, seaweed-like aroma which is variously modified to yield the aromas that are recognizable for individual species. Little has been reported on the identification of compounds characterizing fresh fish aroma; however, Geiselman (1972) studied rainbow smelt (*Osmerus mordax*) and observed compounds reminiscent of cucumbers, violets, and rushes but was unable to make positive identifications. Recently, Berra et al. (1982) identified (*E,Z*)-2,-6-nonadienal as a fresh cucumber-like aroma compound associated with Australian grayling (*Prototroctes maraena*) and Whitfield et al. (1981, 1982) identified (*Z*)-1,5-octadien-3-ol and 1-octen-3-ol as volatile compounds important to the metallic off-flavor of deep sea prawn (*Hymenopenaeus sibogae*) and sand lobster (*Ibacus peronii*).

During studies on the flavor stability of frozen whitefish (*Coregonus clupeaformis*), it was necessary to establish the chemical nature of fresh whitefish aroma. These freshwater fish are characterized by a distinct melon- or cucumber-like aroma, and the identification of the family of carbonyl compounds responsible for this characteristic aroma as well as another family of carbonyl compounds is reported herein. Previously these compounds have been associated principally with the enzymically formed flavors of melons and mushrooms, and their selective occurrence is fish is novel.

MATERIALS AND METHODS

Fresh (36 h on ice), round Lake Michigan whitefish (*C. clupeaformis*) were obtained from a commerical fish source (Green Bay, WI). Fish were eviscerated, washed, drained,

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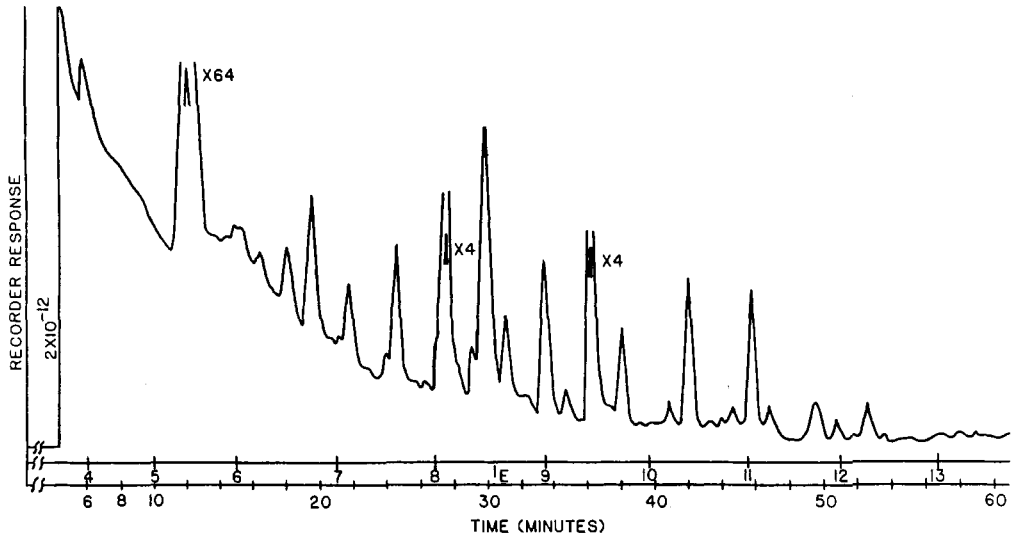

Figure 1. Gas chromatogram of fresh Lake Michigan whitefish (*C. clupeaformis*) headspace volatiles using a packed Carbowax 20 M column.

vacuum packed (>28 in Hg) in Freshtuff (American Can Co., Neenah, WI) barrier bags, and stored at −25 °C until analyzed.

Approximately 100 mL of saturated NaCl solution was added to each fish in a polyethylene bag and agitated for 1 min to facilitate removal of the slime layer. Then 99 mL of the extract was added to 1 mL of an internal standard solution (active amyl alcohol) in a 100-mL round-bottom flask and purged with nitrogen for 2.5 h at 60 mL/min at room temperature (21 °C). Active amyl alcohol (3-methyl-1-butanol; Aldrich Chemical Co., Milwaukee, WI) was selected as the internal standard for quantitative estimation of volatile compound because it eluted in an uncluttered region of the chromatogram. It was added at a level of 1.25 ppm. Volatiles were trapped with Tenax GC (60–80 mesh, product of ENKA N.V., Holland) as described by Steinke (1978). Subsequently, volatiles were eluted with ethyl ether by using 0.1 mL of solvent, and concentration was achieved under a stream of nitrogen. The resulting concentrate exhibited the characteristic aroma of fresh whitefish.

Ethyl ether extracts were separated with a Varian 1740 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with an effluent splitter (4:1 in favor of the exit port) and a flame ionization detector (FID). The effluent splitter allowed simultaneous FID and odor evaluations of eluting compounds. Separations were carried out with packed and glass capillary columns. A 3 m × 2 mm i.d. silane deactivated glass column packed with 9% Carbowax 20 M on 80–100-mesh Chromosorb W AW/DMCS, programmed from 50 to 225 °C at 2 °C/min, was used for packed column analysis. Nitrogen carrier gas, hydrogen, and air flow rates were 24, 24, and 240 mL/min, respectively, and injection port and detector temperatures were 250 and 235 °C, respectively.

Capillary column analyses were carried out in conjunction with mass spectrometric analysis (GC–MS) which was performed with a Finnigan 4021 GC–MS fitted with a PPINICI (pulsed positive ion negative ion chemical ionization) analyzer and an INCOS 2300 data system (Finnigan Instruments, Sunnyvale, CA). Ion source operating temperatures were maintained at 250 °C (EI) or 150 °C (CI) and ionization voltage was 70 eV during scanning. Capillary column analyses were made with splitless injection (activated 0.5 min after injection) on a Carbowax 20 M (60 m × 0.31 mm i.d.) fused silica column (J & W Scientific, Inc., Rancho Cordova, CA) using helium carrier gas (head pressure 10 psi, split 50 mL/min, sweep 5 mL/min) and a program rate of 5 °C/min from 50 to 100 °C followed by from 100 to 220 °C at 10 °C/min. Source pressure (isobutane) was 300 μm for CI determinations.

Compounds were identified by computer matching of full or partial mass spectra of compounds published in "EPA/NIH Mass Spectral Data Base" (Heller and Milne, 1975, 1980) and manual matching with published spectral data. Coincidence of retention indices (I_E; Van den Dool and Kratz, 1963) for unknown and authentic compounds, and where possible agreement of aromas of eluting unknown compounds, was also employed in assigning identities.

RESULTS AND DISCUSSION

The aromas of whitefish flesh and whitefish in the round are very similar and each contains the same volatile compounds. However, the slime and juices contain higher concentrations of compounds and provide a more convenient source of volatiles than muscle tissue. Therefore, this medium was chosen as the source of volatile compounds associated with fresh uncooked whitefish. A typical packed column chromatogram of the volatile compounds associated with fresh whitefish that was obtained by the headspace collection on Tenax GC is shown in Figure 1. Enzymatic and microbial activity during the collection period was minimized through the use of saturated NaCl as the extracting medium, thus reducing the possibility of generating flavor volatiles while purging. Retention of volatile compounds in ethyl ether extracts necessitated nitrogen stripping at room temperature (21 °C) to a final volume of no less than 10 μL. So that efficient detector response could be achieved, 6–7 μL injections of the concentrate onto the packed column was required, and this gave a large, tailing, solvent peak. As a result lower molecular weight compounds were not observed or evaluated.

The compounds identified in fresh whitefish are listed in Table I, and a corresponding typical capillary column GC separation is shown in Figure 2. Notably, two families of compounds previously associated with cucurbits and basidiomycetes were identified. Compounds responsible for the distinct cucumber-like top note in whitefish were identified as (*E*)-2-nonenal (5.8 ± 1.5 ppb; threshold (*T*) = 0.08 ppb; peak 33) and (*E,Z*)-2,6-nonadienal (17.2 ± 0.1 ppb; *T* = 0.01 ppb; peak 36), and 6-nonen-1-ol (4.8 ± 0.8

Table I. Volatile Compounds Identified in Fresh Whitefish (*C. clupeaformis*)

peak no.	compounds	I_E	estimated concn,[a] ppb	GC effluent odor quality (packed column)	odor threshold,[b] ppb	identification means
1	2,3-butandione	3.16	1.7	butter-like	8.6 a	MS, Rt, odor
2 XO[c]	hexanal	4.49	20.5 ± 9.2	green	4.5 a	MS, Rt, odor
3	1-penten-3-ol	5.08	9.5 ± 2.1		400 a	MS, Rt
4	3-heptanone	5.20	4.0	earthy		MS, Rt
5	2-methyl-2-pentenal	5.24	1.95 ± 0.19			MS, Rt
6	2-heptanone	5.40	3.2 ± 3.1		300 a	MS, Rt
7	dodecane	5.50	0.6 ± 0.1			MS, Rt
8	heptanal	5.51	1.8 ± 0.6		3.0 a	MS, Rt
9	3-methyl-1-butanol	5.61	internal standard (1.25 ppm)			MS, Rt, odor
10 O	(*E*)-2-hexenal	5.83	1.4 ± 0.6		17 a	MS, Rt
11	1-pentanol	6.03	1.6 ± 0.2		120 a	MS, Rt
12 X	3-octanone	6.18	2.0 ± 1.1		50 d	MS, Rt
13	ethenylbenzene	6.26	1.4			MS, Rt
14 O	trimethylbenzene	6.40	3.4 ± 0.2			MS,
15	acetoin	6.46	not measured			MS, Rt
16	2-octanone	6.52	1.0 ± 0.1	peaks 16 and 17—green, earthy, aldehyde	150 a	MS, Rt
17	octanal	6.57	0.6 ± 0.1		0.7 a	MS, Rt
18 X	1-octen-3-one	6.67	2.6 ± 1.5	boiled mushrooms	0.09 a	MS, Rt, odor
19	tridecane	6.71	0.8 ± 0.2			MS, Rt
20	2,3-octanedione	6.89	2.0 ± 1.6			MS, Rt
21	1-hexanol	7.14	1.6 ± 0.8		4870 a	MS, Rt
22 X	1,5-octadien-3-one	7.42	1.3 ± 0.3	geranium leaves	0.001 c	MS, Rt, odor
23 X	3-octanol	7.54	1.0 ± 0.6		18 d	MS, Rt
24 O	nonanal	7.62	3.2 ± 1.1	planty, aldehyde	1.0 a	MS, Rt, odor
25	tetradecane	7.73	0.8 ± 0.2			MS, Rt
26 X	(*Z*)-2-octenal	7.91	1.4 ± 0.3	peaks 26 and 27—fatty, heavy, green	3.0 d	MS, Rt
27	acetic acid	7.95	4.5 ± 0.6		7.0 a	MS, Rt
28 X	1-octen-3-ol	8.12	18.6 ± 4.1	raw mushrooms	10 d	MS, Rt, odor
29 X	1,5-octadien-3-ol	8.45	24.8 ± 2.0	earthy, mushroom	10 e	MS, Rt, odor
30 XO	benzaldehyde	8.69	1.0	peaks 30, 31, and 32—slight cucumber, green, vine-like	0.44 a	MS, Rt
31 O	decanal	8.69	2.6 ± 0.4		0.1 a	MS, Rt
32	pentadecane	8.75	2.4 ± 0.2			MS, Rt
33 O	(*E*)-2-nonenal	9.00	5.8 ± 1.5	cardboard-like, cucumber	0.08 a	MS, Rt, odor
34 X	1-octanol	9.11	0.5		480 d	MS, Rt
35	2,3-butandiol	9.15	6.4 ± 2.6			MS, Rt
36 O	(*E,Z*)-2,6-nonadienal	9.43	17.2 ± 0.1	cucumber rind, peeling	0.01 b	MS, Rt, odor
37 X	2-octen-1-ol	9.72	6.3 ± 0.4	peaks 37 and 38—green, musty	40 d	MS, Rt, odor
38	hexadecane	9.74	1.4 ± 0.2			MS, Rt
39 O	1-nonanol	10.19	0.8			
40 X	2,5-octadien-1-ol	10.34	3.8 ± 1.5	peaks 40 and 41—fresh fish undertone		MS, Rt
41 O	6-nonen-1-ol	10.41	4.8 ± 0.8			MS, Rt
42	heptadecane	10.67	2.0 ± 1.0			MS, Rt
43 O	3,6-nonadien-1-ol	11.05	5.7 ± 0.7	clean cucumber	10 b	MS, Rt
44	naphthalene	11.14	1.3 ± 0.2			MS, Rt
45	octadecane	11.62	1.6 ± 0.7			MS, Rt
46	hexanoic acid	11.82	1.1			
47	unknown	12.38	12.4 ± 4.1	green, cucumber-like		43 (100), 71 (60), 41 (35), 56 (32), 55 (30), 83 (21), 39 (14), 89 (12), 98 (10)

[a] Based on duplicate determinations; μg/L slime-water extract. [b] Threshold in water: (a) Frazzalari (1978); (b) Buttery (1981); (c) Swoboda and Peers (1977); (d) Pyysalo and Suihko (1976); (e) Whitfield et al. (1982). [c] X = previously identified in mushrooms. O = previously identified in cucumber/melon.

ppb; T = ? ppb; peak 41) was also probably above the recognition threshold concentration. 3,6-Nonadien-1-ol (5.7 ± 0.7 ppb; T = 10 ppb; peak 43) was found but appeared below the reported recognition threshold concentration. An unidentified compound (peak 47) possessing a green cucumber-like aroma was detected at concentrations of about 12 ppb. Mass spectra suggest this component to be structurally related to 2-octenal and 1,5-octadien-3-ol [*m/e* 43 (100), 71 (60), 41 (35), 56 (32), 55 (30), 83 (21), 39 (14), 89 (12), and 98 (10)], but the retention index on Carbowax 20 M suggests that this compound is near a chain length of 12 carbons. However, definitive CI spectra were not obtained.

Volatile C9 aldehydes and alcohols have been reported as contributing to the odor of cucumber (Forss et al., 1962; Kemp et al., 1974a) and melons (Kemp et al., 1973, 1974b). The odor of fresh cucumber has been attributed largely to (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal (Forss et al., 1962). Kemp et al. (1972) found (*Z*)-6-nonen-1-ol has an odor reminiscent of cucumber and also described (*Z,Z*)-3,6-nonadien-1-ol as reminiscent of watermelon rind (Kemp et al., 1974b).

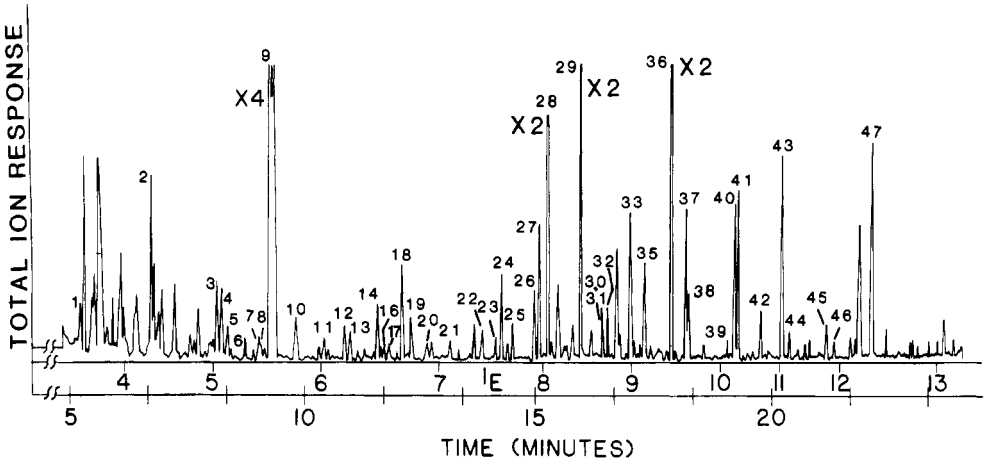

Figure 2. Gas chromatogram of fresh Lake Michigan whitefish (*C. clupeaformis*) headspace volatiles using a fused silica 60 m Carbowax 20 M capillary column. The identity of each numbered peak is listed in Table I.

Although fresh whitefish does not exhibit a distinct mushroom-like aroma, the eight-carbon ketones and alcohols previously associated with mushroom odor (Pyysalo and Suihko, 1976; Maga, 1981; Tressel et al., 1982) contribute a heavy, planty aroma to the overall fresh fish aroma blend. Among those compounds identified in whitefish, 1-octen-3-ol (18.6 ± 4.1 ppb; T = 10 ppb; peak 28), 1-octen-3-one (2.6 ± 1.5 ppb; T = 0.09 ppb; peak 18), 1,5-octadien-3-ol (24.8 ± 2.0 ppb; T = 10 ppb; peak 29), 1,5-octadien-3-one (1.3 ± 0.3 ppb; T = 0.001 ppb; peak 22), 2-octen-1-ol (6.3 ± 0.4 ppb; T = 40 ppb; peak 37), and 2,5-octadien-1-ol (3.8 ± 1 .5 ppb; T = ? ppb; peak 40) appear to have the most potential for influencing fresh fish aromas. If it is assumed that mono- and diunsaturated alcohols follow the same threshold pattern as the mono- and diunsaturated ketones, as is suggested by 1-octene-3-ol and 1,5-octadien-3-ol, 2,5-octadien-1-ol would be expected to occur above threshold concentration. The geometric configuration of compounds identified in whitefish, previously associated with mushrooms, was not determined, but they are believed to exist in the cis (Z) form. Of the 13 compounds reported in mushroom aroma by Tressel et al. (1982), 12 were identified in fresh whitefish. (Z,Z)-2,5-Octadienal was the only compound previously associated with mushrooms that was not identified in fresh whitefish.

During packed column separations, a peak possessing a fresh fish undertone was noted. Upon more efficient separation using a 60-m capillary column, it was found that this peak was the result of two coeluting compounds. These two compounds, 2,5-octadien-1-ol (peak 40) and 6-nonen-1-ol (peak 41) separately possess mushroom-like and cucumber odors, respectively. It appears that the blending of these two families of compounds previously discussed with regards to cucurbits and basidiomycetes contributes strongly to the aroma associated with fresh whitefish. Similar observations for combined aroma effects of a decatrienal and the decadienals were reported by Swoboda and Peers (1977).

Trimethylbenzene (isomer, peak 14) and benzaldehyde (peak 30) have been reported in melon fruit (Kemp et al., 1972) while benzaldehyde has been reported in mushrooms (Tressel et al., 1982). Although their origin in foods has not been established, their discovery in fresh fish suggests a common biochemical mechanism(s).

Microbial involvement in the production of the characterizing carbonyls of fresh fish appears unlikely at this time. When the described headspace collection technique was used, microbially produced volatiles, 2,3-butandione, acetoin, acetic acid, and 2,3-butandiol, were identified. However, these traditional microbial metabolites were present at low concentrations, which indicated limited microbial activity in samples analyzed.

The formation of volatile aldehydes and alcohols in plant materials by enzymatic oxidation of lipids following tissue disruption has been studied extensively (Zimmerman and Vick, 1973; Galliard, 1975; Wardale and Galliard, 1975; Galliard and Phillips, 1976; Grosch and Laskawy, 1975; Vick and Zimmerman, 1976; Bonnet and Crouzet, 1977; de Lumen et al., 1978; Tressel et al., 1981). Lipoxygenase, distributed widely among plants, catalyzes the hydroperoxidation of polyunsaturated fatty acids having a (Z,Z)-1,4-pentadiene system (Gardner, 1975). On the basis of early research, Tappel (1961) concluded that lipid peroxidation in meats and fish could not result from lipoxygenase activity. The peroxidation of lipids in nonliving animal tissue reportedly is initiated nonenzymatically, largely by hemoproteins (Gardner, 1975). However, the similarity between the newly identified fresh fish volatiles and those identified earlier in melon fruits and mushroom strongly suggests a lipoxygenase-like enzyme system in fish. The possible existence of a biosynthetic mechanism largely residing in the skin that is capable of catalyzing the production of characterizing fresh fish aroma volatiles will be the subject of a following paper.

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Registry No. **1**, 431-03-8; **2**, 66-25-1; **3**, 616-25-1; **4**, 106-35-4; **5**, 623-36-9; **6**, 110-43-0; **7**, 112-40-3; **8**, 111-71-7; **9**, 123-51-3; **10**, 6728-26-3; **11**, 71-41-0; **12**, 106-68-3; **13**, 100-42-5; **14**, 25551-13-7; **15**, 513-86-0; **16**, 111-13-7; **17**, 124-13-0; **18**, 4312-99-6; **19**, 629-50-5; **20**, 585-25-1; **21**, 111-27-3; **22**, 65213-86-7; **23**, 589-98-0; **24**, 124-19-6; **25**, 629-59-4; **26**, 20664-46-4; **27**, 64-19-7; **28**, 3391-86-4; **29**, 83861-74-9; **30**, 100-52-7; **31**, 112-31-2; **32**, 629-62-9; **33**, 18829-56-6; **34**, 111-87-5; **35**, 513-85-9; **36**, 557-48-2; **37**, 22104-78-5; **38**, 544-76-3; **39**, 143-08-8; **40**, 83861-75-0; **41**, 40709-05-5; **42**, 629-78-7; **43**, 76649-25-7; **44**, 91-20-3; **45**, 593-45-3; **46**, 142-62-1.

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