obtained from commercial sources unless otherwise reported.

Method A—A mixture of a ketone (0.10 mole), 2,6-dimethylmorpholine hydrochloride (0.10 mole), paraformaldehyde (0.20 mole), and concentrated hydrochloric acid (0.50 ml.) in 50 ml. of absolute ethanol was heated under reflux. After refluxing for 3 hr., another 0.10 mole of paraformaldehyde was added and refluxing continued for an additional 6 hr. Boiling acetone (200 ml.) was added to the hot mixture with shaking. The resulting solution was allowed to cool overnight, and then in the refrigerator for 3 hr. The crystalline product thus precipitated was filtered. Ethanol–acetone is a suitable solvent pair for crystallization.

Method B—The β-amino acids were prepared according to the procedure of Mannich and Stein (4). A white solid started separating after about 4 hr. The crystalline product thus precipitated after 24 hr. was filtered. The residue was washed twice with absolute ethanol (50-ml. portions) and dried. Some of these acids turn red on standing, probably due to air oxidation.

Method C—Thionyl chloride (20 ml.) was added to the β-amino acid (0.025 mole) dropwise with cooling and shaking. After the completion of addition, the mixture was allowed to stand at room temperature for about 16 hr. During this period, the entire solid went into solution. The unreacted thionyl chloride then was removed on the evaporator. The residue then was taken up in carbon tetrachloride (100 ml.), methanol (20 ml.) was added slowly, and the resulting mixture was refluxed on a steam bath. After about 6 hr., the solvent and unreacted methanol were removed on the evaporator, and a portion of the resulting residue was recrystallized from acetone.

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Stability of Metal Complexes of Salicylic Acid Derivatives and Analogs III

3,6-Dialkyl Derivatives and Pyridine Analogs

By WILLIAM O. FOYE, MARTIN D. BAUM, and DAVID A. WILLIAMS

Stability constants are reported for the complexation of a series of 3,6-dialkylsalicylic acids and pyridine analogs of salicylic acid with Cu (II), Fe (III), and Al (III) ions. These compounds were selected in an attempt to increase the affinity of salicylates for ferric ion. The 3,6-dialkyl substituents lowered metal complex stabilities, but the presence of a ring-nitrogen adjacent to the phenolic group increased complex stabilities considerably. The 3,6-dialkylsalicylic acids revealed somewhat greater analgesic effects than salicylic acid, but less anti-inflammatory action.

THE AVIDITY of salicylic acid for complex formation with transition metals has suggested that some, if not all, of the biological effects of salicylates may be due to complexation of metalloenzymes (1). Ample evidence may be found in the literature that salicylic acid or its derivatives become involved in enzymatic reactions; some references of this nature were cited in a previous paper (1), and other examples have appeared since (2–6). Since salicylates apparently affect cellular oxidations, salicylate derivatives which show a greater affinity for iron might be expected to show enhanced biological effects. Increased affinity for iron could be realized in two ways. Introduction of bulky substituents in the 3,6-positions of salicylic acid would tend to crowd the chelating groups together and thus provide a better fit for the ferric ion, which is a relatively small ion (7). Or the introduction of nitrogen into the ring in positions adjacent to the chelating groups might also provide molecules

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with an increased affinity for iron, which has a greater affinity for nitrogen than oxygen (7).

Both types of salicylate derivatives were therefore prepared, and their complex forming abilities with Fe (III), Al (III) (a still smaller ion), and Cu (II) (a larger ion, but also involved in cellular oxidations) ions have been determined. Some of the characteristic biological effects of salicylates were then determined, and possible correlations with metal-binding strengths were sought. In this way, evidence for a possible mode of salicylate action might be obtained, or more potent salicylate derivatives found.

METHODS

Analytical reagent grade $Fe(NO_3)_3 \cdot 9H_2O$, $AlCl_3 \cdot 6H_2O$, and $CuCl_2 \cdot 2H_2O$ were used, and carbonate-free 0.01 *M* sodium or potassium hydroxide solution was prepared according to Armstrong (8).

The solutions were stored in polyethylene bottles under nitrogen and diluted quantitatively with boiled distilled water just prior to use. The normality was checked against potassium diphthalate. The organic ligands were prepared as described below, and their purity was ascertained by thin-layer chromatography. The method of Reio (9) was used, in which the developing solvent contained methyl ethyl ketone, acetone, formic acid, and water in the ratio of 40:2:1:6. The plates were sprayed with a solution of bromophenol blue in ethanol (0.06%) containing 1 drop of 10% sodium hydroxide solution, and the presence of one spot confirmed the absence of isomers or other impurities.

Melting ranges were determined with a Mel-Temp apparatus and are corrected. Elemental analyses were obtained from Weiler and Strauss, Oxford, England. 3,4-Pyridinedicarboxylic acid was obtained from Aldrich Chemical Co. and was recrystallized to constant melting point (260°) from water.

2 - Hydroxy - 3,6 - dimethylbenzoic Acid—The method of Moody (10) was modified. 2,5-Dimethylphenol (25 Gm., 0.204 mole) dissolved in 200 ml. of xylene was placed in a 1000-ml. Parr autoclave. Granular sodium (9.5 Gm., 0.41 mole) was added and the bomb was sealed and heated at 160° for 3 hr. Xylene (300 ml.) and dry ice (50 Gm.) were added to the cooled contents, and the bomb was resealed and heated at 160° for 10 hr. with periodic shaking. Water (300 ml.) was then cautiously added to the cooled contents, and the mixture was heated, cooled, and separated. The aqueous layer was acidified, and the resulting precipitate was collected and dried. The crude product was purified by complexation with copper sulfate in aqueous solution, filtering, acidifying the filtrate, and collecting the precipitate. The yield was 24 Gm. (90%), m.p. 197–198°. [Lit. m.p. 193° (10).] Further purification resulted by dissolving the product in aqueous sodium bicarbonate, boiling with activated charcoal, filtering, acidifying, and collecting the precipitate, m.p. 200–201°.

Anal.—Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.08. Found: C, 65.36; H, 6.41.

2 - Hydroxy - 3 -methyl - 6 - isopropylbenzoic Acid—A modification of the method of Sondern (11) was used. 2-Methyl-5-isopropylphenol (50 Gm., 0.33 mole) was dissolved in 300 ml. of xylene, which was stirred and refluxed, and carbon dioxide was admitted continuously. Granular sodium (15.3 Gm., 0.67 mole) was added during 1 hr., and the mixture was stirred for 5 hr. and cooled. Water was then added slowly until all sodium was spent. The mixture was heated, cooled, and separated, and the aqueous layer was acidified. The precipitate was collected, dissolved in hot alcohol, and the phenol was removed by addition of water. The hot supernate was allowed to cool, and crystals were collected and dried. After recrystallization from ethanol–acetic acid (1:1), a 16% yield was obtained, m.p. 157–158°. [Lit. m.p. 134° (12).]

Anal.—Calcd. for $C_{11}H_{14}O_3$: C, 68.10; H, 7.22 Found: C, 67.81; H, 7.22.

2 - Hydroxy - 3 - tert - butyl - 6 - methylbenzoic Acid—The above procedure was followed, and from 50 Gm. (0.304 mole) of 2-*tert*-butyl-5-methylphenol was obtained a 19% yield of product, m.p. 183–184°. [Lit. m.p. 182° (10).]

Anal.—Calcd. for $C_{12}H_{16}O_3$: C, 69.28; H, 7.75. Found: C, 69.09; H, 7.69.

2 - Hydroxy - 3,6 - dimethyl - 5 - nitrobenzoic Acid—Ten grams (0.06 mole) of 2-hydroxy-3,6-dimethylbenzoic acid was nitrated according to the method of Sondern (11). The crude product was washed several times with ethylene dichloride, dissolved in aqueous sodium bicarbonate, boiled with activated charcoal, filtered, acidified, and collected. A 16% yield was obtained, m.p. 202–205° dec. [Lit. m.p. 182–184° dec. (10).]

Anal.—Calcd. for $C_9H_9NO_5$: C, 51.30; H, 4.26; N, 6.64. Found: C, 51.36; H, 4.47; N, 6.70.

2 - Hydroxy - 3,6 - dimethyl - 5 - aminobenzoic Acid Hydrochloride—The previous product (1 Gm., 0.0047 mole) was dissolved in absolute ethanol (50 ml.) in a hydrogenation bottle, and 1.1 equivalents of dry hydrogen chloride were added. Ten per cent palladium-on-charcoal (0.5 Gm.) was added, and the compound was hydrogenated at 2–3 atm. After reduction was complete, the catalyst was removed, and the alcohol was evaporated. The residue was washed with ethyl acetate, dissolved in hot glacial acetic acid, and precipitated with benzene. The resulting crystals (0.4 Gm., 40%) melted at 245–246° dec.

Anal.—Calcd. for $C_9H_{12}ClNO_3$: C, 49.66; H, 5.56; N, 6.44. Found: C, 49.80; H, 5.27; N, 6.18.

2 - Hydroxypyridine - 3 - carboxylic Acid—The method of Feibel and Spoerri (13) was used. The product melted at 256–257°. [Lit. m.p. 255° (13).]

Anal.—Calcd. for $C_6H_5NO_3$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.32; H, 3.75; N, 10.20.

3 - Hydroxypyridine - 4 - carboxylic Acid—The method of Bachman and Barker (14) was used. The product melted at 315–316° dec. [Lit. m.p. 315° dec. (14).]

Anal.—Calcd. for $C_6H_5NO_3$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.52; H, 3.90; N, 10.26.

Ionization Constants—The methods employed were a spectrophotometric procedure for groups having a pKa value below 2 or above 11 and a potentiometric procedure for groups having a pKa

value between 2 and 11. Both methods are fully described by Albert and Serjeant (15).

Stability Constants—Potentiometric titrations were carried out under nitrogen either in 95% ethanol or in distilled water. Fifty-milliliter volumes of 0.001 *M* solutions of the organic acid were titrated with 0.01 *N* sodium or potassium hydroxide in 0.5-ml. portions first in the absence of metal ions and then in the presence of 0.000025 mole of a divalent metal salt or 0.0000167 mole of a trivalent metal salt. Fifty-milliliter volumes of the same quantities of the metal salts were also titrated with 0.01 *N* alkali. The aqueous solutions titrated were first brought to a constant ionic strength with 0.1 *M* potassium chloride. In several instances, titration with alkali did not uncover all the values necessary for calculation of the constants, so resort was made to back titration with 0.01 *N* hydrochloric acid.

In the case of the titrations done in 95% alcohol, pH values were recorded with a Vibron Electrometer (model 33B, Electronic Instruments Ltd.) with a pH attachment and glass (type GG 33) and calomel (type RJ 23) electrodes, as described by Albert and Serjeant (16). After each addition of alkali, the solution was mixed for 2 min. before the reading was made. For the titrations in water, pH values were measured with a Beckman research pH meter using glass and calomel electrodes.

When 3-hydroxypyridine-4-carboxylic acid was mixed with cupric chloride in the above concentrations, precipitation occurred. This required a 0.0002 *M* solution of the acid and 0.0001 *M* solution of cupric chloride to be titrated with 0.002 *N* alkali.

Calculations were done as previously (17), except that $[L^-]$ has been substituted for [Sc] (concentration of free chelating species), βnm for Ks, and P and Q for β and α, respectively, in conformance with present convention. This gives the following equations for the solution of values of $[L^-]$:

$$\log [L^-] = \log([Lo] - [KOH] - [H^+] + [OH^-]) - \log P \quad \text{(Eq. 1)}$$

$$\log [L^-] = \log(2[Lo] - [KOH] - [H^+] + [OH^-]) - \log P \quad \text{(Eq. 2)}$$

The term $[OH^-]$ was generally dropped, since the titrations were not usually carried to a pH of 10, and the term was therefore insignificant. Values of $\bar{n}$ were calculated from:

$$\bar{n} = \frac{[Lo] - Q[L^-]}{[Mo]} \quad \text{(Eq. 3)}$$

If the pH in the presence of metal ion more closely approximated the pH value obtained with the organic acid alone at one equivalent, then Eq. 1 was used; at two equivalents, Eq. 2. Equation 1 was used for the 2-hydroxypyridine-3-carboxylic acid systems, where one proton was apparently picked up by the ring nitrogen, and only one proton was liberated; Eq. 2 was used for the others. Formation curves were also plotted ($\bar{n}$ against $-\log [L^-]$) to show whether stepwise complexation had taken place (18).

Values of K_1, K_2, and K_3 were calculated as before (17) where the difference between log K_1 and log K_2 was at least 2.5. In those cases where the values of log K_1 and log K_2 were closer, K_1 and β_2 were evaluated from Eq. 4 using the method of least squares (15).

$$\frac{\bar{n}}{(\bar{n} - 1)[L^-]} = \frac{(2 - \bar{n})[L^-]}{(\bar{n} - 1)} \beta_2 - K_1 \quad \text{(Eq. 4)}$$

RESULTS AND DISCUSSION

Table I lists the ionization constants determined for the salicylate analogs and derivatives, and Tables II–IV list the stability constants for the complexes with Cu (*II*), Fe (*III*), and Al (III) ions. The values of the stability constants obtained in aqueous alcohol (final concentration approximately 79% ethanol) cannot be considered valid for some purposes, but for comparison of metal-binding abilities with biological effects, these values should suffice. (For a discussion of the irregularities in ionization constants, and consequently in metal-binding constants, to be expected in nonaqueous or mixed solvents, see *Reference 15*, pp. 66–68.) However, the same sequence of complexing abilities should be found in water as in aqueous alcohol. The complexes of these dialkyl-substituted salicylic acids were too insoluble in water to permit pH measurements in meaningful concentrations, despite the use of the more sensitive instrument employed.

Not all values of K_1, K_2, or K_3 could be obtained by titration with alkali, either because of precipitation of the metal complexes, or because in several cases values of $\bar{n}$ less than 1 were not realized. In the latter cases, high affinity of the metal for the ligand probably prevented part of the titration curve from being revealed, and no complexation was evident until one equivalent of alkali had been added (as in the case of the cupric complexes of the 3,6-dimethyl and 5-nitro-3,6-dimethyl derivatives in water). The latter situation was corrected by back titration with acid.

TABLE I—IONIZATION CONSTANTS (25°)

Acid	Solvent	pKa¹	pKa²	pKa³
2-Hydroxy-3,6-dimethylbenzoic	EtOH	6.07	13.20	
2-Hydroxy-3-*tert*-butyl-6-methylbenzoic	EtOH	5.77	13.05	
2-Hydroxy-3-methyl-6-isopropylbenzoic	EtOH	5.70	13.00	
2-Hydroxy-5-amino-3,6-dimethylbenzoic	EtOH	6.37	11.55	
2-Hydroxybenzoic[a]	H_2O	3.00	13.82	
2-Hydroxy-3,6-dimethylbenzoic	H_2O	3.23	12.32	
2-Hydroxy-5-nitro-3,6-dimethylbenzoic	H_2O	2.78	8.54	
2-Hydroxypyridine-3-carboxylic[b]	H_2O	3.28	5.33	12.62
3-Hydroxypyridine-4-carboxylic	H_2O	0.10	4.83	11.30
3,4-Pyridinedicarboxylic	H_2O	1.50	2.95	5.07

[a] *Reference 15*, p. 134. [b] Canic, V., *et al.*, *Glasnik Khem. Drushtva Beograd*, **21**, (No. 2) 65(1956); through *Chem. Abstr.*, **54**, 8 15(1960).

TABLE II—STABILITY CONSTANTS OF THE CUPRIC COMPLEXES (25°)

Acid	Solvent	Log K_1	Log K_2	Log β_2
2-Hydroxy-3,6-dimethylbenzoic	EtOH–H_2O	13.93	9.48	23.41
2-Hydroxy-3-*tert*-butyl-6-methylbenzoic	EtOH–H_2O	13.21	9.82	23.03
2-Hydroxy-3-methyl-6-isopropylbenzoic	EtOH–H_2O	12.66	8.22	20.88
2-Hydroxy-5-amino-3,6-dimethylbenzoic	EtOH–H_2O	10.51		
2-Hydroxybenzoic[a]	0.1 *M* KCl	10.60	7.85	18.45
2-Hydroxy-3,6-dimethylbenzoic	0.1 *M* KCl	9.06		17.83
2-Hydroxy-5-nitro-3,6-dimethylbenzoic	0.1 *M* KCl	5.87	3.55	9.42
2-Hydroxypyridine-3-carboxylic	0.1 *M* KCl	18.29	15.04	33.33
3-Hydroxypyridine-4-carboxylic	0.1 *M* KCl	9.98		18.47
3,4-Pyridinedicarboxylic	0.1 *M* KCl	3.07		6.52

[a] Sillén, L. G., and Martell, A. E., "Stability Constants of Metal-Ion Complexes," The Chemical Society, Burlington House, London, England, 1964, p. 534.

TABLE III—STABILITY CONSTANTS OF THE FERRIC COMPLEXES (25°)

Acid	Solvent	Log K_1	Log K_2	Log K_3	Log β_3
2-Hydroxy-3,6-dimethylbenzoic	EtOH–H_2O	16.93	14.83		
2-Hydroxy-3-*tert*-butyl-6-methylbenzoic	EtOH–H_2O	16.17	13.86		
2-Hydroxy-3-methyl-6-isopropylbenzoic	EtOH–H_2O	15.57	13.89		
2-Hydroxy-5-amino-3,6-dimethylbenzoic	EtOH–H_2O	12.26	9.40		
2-Hydroxybenzoic[a]	0.1 *M* KCl	16.48	11.68	8.68	36.84
2-Hydroxy-3,6-dimethylbenzoic	0.1 *M* KCl	14.76	11.70		
2-Hydroxy-5-nitro-3,6-dimethylbenzoic	0.1 *M* KCl	8.98	8.31	6.04	23.33
2-Hydroxypyridine-3-carboxylic	0.1 *M* KCl	17.53	15.50		
3-Hydroxypyridine-4-carboxylic	0.1 *M* KCl	11.97	9.16		
3,4-Pyridinedicarboxylic	0.1 *M* KCl	5.68	4.55		

[a] Sillen, L. G., and Martell, A. E., *op. cit.*

TABLE IV—STABILITY CONSTANTS OF THE ALUMINUM COMPLEXES (25°)

Acid	Solvent	Log K_1	Log K_2	Log K_3	Log β_3
2-Hydroxy-3,6-dimethylbenzoic	EtOH–H_2O	16.12	14.67		
2-Hydroxy-3-*tert*-butyl-6-methylbenzoic	EtOH–H_2O	15.12	13.97	11.85	40.94
2-Hydroxy-3-methyl-6-isopropylbenzoic	EtOH–H_2O	15.86	13.89	11.93	41.58
2-Hydroxy-5-amino-3,6-dimethylbenzoic	EtOH–H_2O	12.78	10.65	8.94	32.37
2-Hydroxybenzoic[a]	0.1 *M* KCl	14.00	10.7	8.6	33.3
2-Hydroxy-3,6-dimethylbenzoic	0.1 *M* KCl	11.62	11.06		
2-Hydroxy-5-nitro-3,6-dimethylbenzoic	0.1 *M* KCl	8.64	6.64	4.62	19.90
2-Hydroxypyridine-3-carboxylic	0.1 *M* KCl	16.97	14.90		
3-Hydroxypyridine-4-carboxylic	0.1 *M* KCl	12.00	8.01	7.39	27.40
3,4-Pyridinedicarboxylic	0.1 *M* KCl	4.06	4.02	3.73	11.81

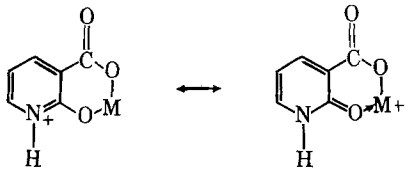
[a] *Reference 17.*

The sequence of complexing abilities found was essentially the same for either the cupric, ferric, or aluminum complexes.

With the 3,6-dialkyl-substituted salicylic acids, the 3,6-dimethyl compound showed more avidity for the metal ions than the more bulky isopropyl or *tert*-butyl derivatives. It also showed less metal-complexing ability in water than salicylic acid itself, so the entire series of 3,6-dialkylsalicylic acids may be considered as having lower complexing ability not only for iron but for the other metal ions observed. Apparently, increased bulk around the chelating carboxyl and hydroxyl functions did not contribute to increased stability, but led instead to increased steric hindrance. Introduction of the 5-amino group also lowered stability, possibly by quinoid contributions by the phenolic group which lowered its basicity. The 5-nitro group lowered stability as would be predicted from its electron withdrawing effect. The presence of a ring-nitrogen atom adjacent to the phenolic group did, however, augment complex stability considerably.

Comparison of the complex stability constants with the ionization constants shows the complex stabilities paralleled by the phenolic ionizations, the greater the basicity of the phenolic group, the greater the stability of the complex. An apparent exception to this may be seen with 2-hydroxypyridine-3-carboxylic acid, where the presence of the ring nitrogen adjacent to the phenolic group would be expected to increase its acidity and thus lower, in some cases, the complex stability constants. The fact that both the phenolic ionization and complex stabilities are greater than those of the 3-hydroxypyridine-4-carboxylic acid suggests that the 2-hydroxy group may undergo tautomerism leading to increased resonance (see structures I and II) and thus increased stability, in the chelate ring. The 3-hydroxy compound is structurally incapable of this tautomerism. That the 5-amino group in the 3,6-dimethylsalicylic acid, also capable of tautomerism, did not show the same enhancement of complex stability may be attributed to the π-excessive character of the 5-amino-substituted ring

and the π-deficient character of the pyridine ring.



I II

Biologic Activities—Results of analgesic testing[1] of the 3,6-dialkyl-substituted salicylic acids showed that both the 3,6-dimethyl and 3-methyl-6-isopropyl derivatives had about 40% greater activity than aspirin, while the 3-*tert*-butyl-6-methyl derivative had about 20% more activity. Analgesic testing was carried out in rats (20 per compound) at compound dosages of 300 mg./Kg. by heating the rat's tail. Because of the lower metal-binding abilities of these acids in water in comparison to that of salicylic acid, it cannot be concluded that greater avidity for metal ions confers greater analgesic effects. It is significant, however, that *o*-thymotic acid also claimed to have a greater analgesic effect than salicylate (19) has also somewhat lower metal-binding constants (1).

Tests for anti-inflammatory and antierythemic effects[2] were also positive for 3,6-dimethylsalicylic acid. In this test, carried out by the method of Winder *et al.* (20), a dosage level of 80 mg./Kg. in guinea pigs produced a decrease in response to U.V.-induced erythema in $^7/_8$ animals, compared to the same response from 100 mg./Kg. of aspirin. In comparison, 3,5-diisopropylsalicylic acid (1) reduced these effects in only $^2/_8$ animals, and both 2-hydroxypyridine-3-carboxylic acid and 3-hydroxypyridine-4-carboxylic acid showed no effects at 80 mg./Kg.

[1] Carried out under the direction of Dr. Howard J. Jenkins.
[2] These results were supplied through the courtesy of Dr. Blaine M. Sutton, Smith Kline & French Laboratories.

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Constituents of the Rhizome of *Asarum canadense*

By LUDWIG BAUER, CHARLES L. BELL, JAMES E. GEARIEN, and HIROSHI TAKEDA

A pentane extract from the rhizomes of *Asarum canadense* was separated first by means of steam distillation, and then by column and gas chromatography. From the steam-volatile oil the following constituents were isolated (percentages are shown in parentheses): methyleugenol (44.5), linalyl acetate (41.1), geraniol (7.4), linalool (5.3), limonene (0.8), α-terpineol (0.4), bornyl acetate (0.3), aristolone (0.1), elemicin (0.1), 2,3,4,5-tetramethoxyallylbenzene (0.05), 2,4-dimethoxycinnamaldehyde (0.05), and two unidentified compounds (0.01 each). The steam-nonvolatile residue contained some of the above and also β-sitosterol.

THE FRAGRANT essential oil of *Asarum canadense* (Canada snakeroot, wild ginger) has not been investigated since 1902 when Power reported it to contain mainly methyleugenol (3,4-dimethoxyallylbenzene), pinene, and (after saponification) linalool, borneol, terpineol, geraniol, and several unidentified compounds (1, 2). This work set out to re-examine this oil and, in particular, search for minor constituents. Instead of subjecting the ground rhizomes immediately to steam distillation (1, 2), it was advantageous to obtain a pentane extract first which then was separated into a steam-volatile fraction (oil *A*) and a residue (oil *B*). Examination of oil *A*, by gas chromatography (GC) revealed the presence of at least 13 components (Fig. 1, Table

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