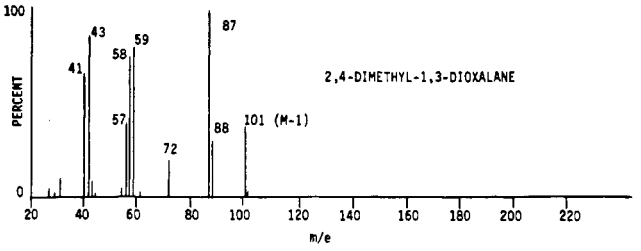

Figure 4. Mass spectra of 2,4-dimethyl-1,3-dioxolane.

Further, the intensity of these three peaks appears in decreasing order of magnitude in the order listed. Neral and geranial are exceptions in that the three peaks appear in the decreasing order m/e 87, 41, 59. The appearance of these three peaks in the mass spectrum serves to identify the presence of 2,4-disubstituted-1,3-dioxolanes since they are specific for this type of compound.

These substituted 1,3-dioxolanes do not appear in all mixtures containing aldehydes and propylene glycol, although it has been noted that the frequency of their appearance in flavors has been increasing. The reason may be due to the increasing use of organic acids as flavorants. However, with the growing availability of mass spectroscopic instrumentation in commercial laboratories the above procedure will provide a rapid means for detection so that corrective action can be taken.

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New Tryptamine Derivatives Isolated from Wax of Green Coffee Beans

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Some unknown 5-hydroxytryptamine derivatives were found to be present in the cuticular wax of green coffee beans. They were isolated by polyamide column chromatography and high-pressure liquid chromatography on a reversed-phase column and were identified by mass spectrometry, infrared spectrometry, proton magnetic resonance, and ultraviolet spectrometry as N_β-(20-hydroxyarachidoyl)-5-hydroxytryptamine and N_β-(22-hydroxybehenoyl)-5-hydroxytryptamine.

Investigation of the chemical composition of the cuticular wax of green coffee beans is part of our research interest (Folstar, 1976). It was previously found that the wax could be separated into two fractions: a fraction which is soluble in petroleum ether (40–60 °C) and a fraction which is insoluble in petroleum ether. The chemical composition of the petroleum ether soluble fraction has been established, and it was found to be largely identical with the composition of coffee bean oil (Folstar et al., 1975a,b). The petroleum ether insoluble part of the wax was fractionated by polyamide column chromatography and high-pressure liquid chromatography (LC); four homologues of N_β-alkanoyl-5-hydroxytryptamine (C-5-HT), in which the alkanoyl group is a stearoyl (C_{18}-5-HT, Ia), arachidoyl (C_{20}-5-HT, Ib), behenoyl (C_{22}-5-HT, Ic), or lignoceroyl (C_{24}-5-HT, Id), were found to be the main constituents; moreover, caffeine and some fractions consisting of unknown compounds were found (Folstar et al., 1979). In this paper we describe the isolation and structural identification of two of these unknown compounds: N_β-(20-hydroxyarachidoyl)-5-hydroxytryptamine (IIa) and N_β-(22-hydroxybehenoyl)-5-hydroxytryptamine (IIb).

EXPERIMENTAL SECTION

Materials. Polyamide-6S for column chromatography was obtained from Riedel de Haën AG, Seelze-Hannover, Germany. Commercially prepared silica gel $60F_{254}$ plates

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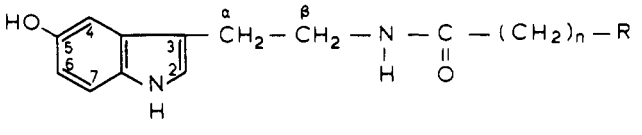

Ia R = CH_3 ; n = 16
Ib R = CH_3 ; n = 18
Ic R = CH_3 ; n = 20
Id R = CH_3 ; n = 22
IIa R = CH_2OH ; n = 18
IIb R = CH_2OH ; n = 20

were obtained from Merck AG, Darmstadt, Germany. LiChrosorb 10RP18 columns for LC were purchased from Chrompack BV, Middelburg, The Netherlands. Green coffee beans (Santos coffee) were kindly supplied by D.E.J. International Research Co., B.V. Utrecht, The Netherlands. Reference samples of 5-hydroxytryptamine and 6-hydroxytryptamine, both as creatinine sulfate complex, were from Sigma, St. Louis, MO.

Isolation and Fractionation of Coffee Wax. Coffee wax was obtained by extracting 10 kg of unground green beans with methylene chloride. The extraction was carried out by heating and stirring portions of 500 g of beans with 1 L of the solvent each, using a 5-L flask which was equipped with a condenser. The flask was heated on a hot plate with a built-in magnetic stirrer. The methylene chloride solutions were combined, and the solvent was evaporated in a rotating vacuum evaporator at 30 °C. Next the residue was extracted with 500 mL of petroleum ether

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Table I. Mass Spectral Data of Unknown Compounds C_1, C_2, and C_3 and Reference Compounds of C_{20}-5-HT[a] and C_{22}-5-HT[a]

m/e (relative intensity)				
C_1	C_2	C_3	C_{20}-5-HT (Ib)	C_{22}-5-HT (Ic)
487 (2)	515 (2)	515 (2)	471 (2)	499 (1)
486 (4)	514 (4)	514 (3)	470 (6)	498 (4)
468 (1)	496 (1)	496 (1)	469 (1)	497 (1)
328 (1)	339 (1)	356 (1)	312 (1)	340 (2)
311 (1)	338 (2)	339 (1)	295 (1)	323 (1)
310 (1)	160 (22)	338 (2)	160 (33)	160 (19)
160 (19)	159 (100)	160 (23)	159 (100)	159 (100)
159 (100)	146 (16)	159 (100)	146 (27)	146 (15)
146 (17)		146 (19)		
molecular ions (calculated mass, molecular formula)				
486.3823 (486.3821, $C_{30}H_{50}N_2O_3$)	514.4136 (514.4134, $C_{32}H_{54}N_2O_3$)	514.4154 (514.4134, $C_{32}H_{54}N_2O_3$)	470.3872 (470.3872, $C_{30}H_{50}N_2O_2$)	498.4176 (498.4185, $C_{32}H_{54}N_2O_2$)

[a] Isolated from wax of green coffee beans (Folstar et al., 1979).

(40–60 °C) four times at 15 min each, using a 1.5-L flask. The extraction was performed as described above. The petroleum ether solution was decanted; the residue constitutes 13 g of petroleum ether insoluble substances of the wax. These substances were fractionated by use of polyamide column chromatography, according to the procedure of Folstar et al. (1979), which was modified slightly. A column of 125 g of polyamide (30 × 4.2 cm) was eluted with 500 mL of benzene, which was continuously mixed successively with 2 L of ether, 2 L of ether–acetone (9:1, v/v), 2 L of ether–acetone (3:1, v/v), 2 L of ether–acetone, (2:1, v/v), 2 L of ether–acetone (1:1, v/v), 5 L of acetone, and 2 L of acetone–ethanol (19:1, v/v). The eluate was collected in 250-mL fractions at a rate of 1 L/h. Next the solvent was evaporated in a rotating vacuum evaporator at 30 °C, and the residue was dissolved in 3–4 mL of chloroform–methanol (1:1, v/v). The composition of the fractions was studied by TLC on silica gel $60F_{254}$ with benzene–acetone–methanol (20:10:0.5, v/v/v) as the solvent and with detection under short-wave UV light and by high-pressure LC under the conditions mentioned below. The fractionation on polyamide was carried out in triplicate, separating one-third of the petroleum ether insoluble substances each time. Most of compounds IIa and IIb eluted after addition of ether–acetone (2:1, v/v) and ether–acetone (1:1, v/v) to the gradient mixer. The fractions with the unknown compounds were combined, the solvent was evaporated, and the residue (1.6 g) was rechromatographed on polyamide under the conditions mentioned above.

High-Pressure Liquid Chromatography of the Unknown Tryptamine Derivatives. The unknown tryptamine derivatives were separated by LC, using a Spectra Physics 3500 B liquid chromatograph equipped with a UV-vis 770 variable wavelength detector. Conditions were as follows: Ten microliters of the sample, dissolved in chloroform–methanol (1:1, v/v), was injected on a 25 × 0.46 cm column with LiChrosorb 10RP18 and methanol–acetonitrile (4:1, v/v) as the mobile phase at 0.4 mL/min; detection was carried out by measuring the extinction of the eluate at 278 nm. The height of the peaks was used as a measure for the concentration. Separations on preparative scale were carried out by use of a 25 × 0.9 cm column with LiChrosorb 10RP18 and methanol–acetonitrile (4:1, v/v) as the mobile phase at 1 mL/min; injections amounted to 250 μL of a 40-mg sample in 10 mL of chloroform–methanol (1:1, v/v).

Spectral Analysis. UV spectra in methanol were recorded with a Varian Cary 118 spectrophotometer. IR spectra were taken on KBr pellets with a Hitachi EPI-G3. ^1H NMR spectra were obtained with a Varian XL100-15 (100 MHz), equipped with a pulse unit and a 620 L-16k computer, in pyridine–carbon tetrachloride (2:3, v/v) and in dimethyl sulfoxide (Me_2SO). Chemical shifts were measured in ppm downfield from internal Me_4Si. Mass spectra were determined with an AEI MS902.

RESULTS AND DISCUSSION

Isolation of Unknown Tryptamine Derivatives. Fractionation of the petroleum ether insoluble part of coffee wax on polyamide gave five peaks (A–E); this is in agreement with results obtained previously (Folstar et al., 1979). Peak A consists of caffeine and peak B was C-5-HT (Ia–Id). By use of LC, peak C was separated into three peaks, C_1, C_2, and C_3, with $k'_{C_1} = 0.9$, $k'_{C_2} = 1.1$, and $k'_{C_3} = 1.4$, respectively ($V_0 = 2.56$ mL); a chromatogram is shown in Figure 1. Peaks D and E were found to be highly complicated; identification was impossible until now.

In order to isolate C_1, C_2, and C_3 for the purpose of identification, the high-pressure liquid chromatographic procedure was repeated on a preparative column with the same column filling material. The peaks C_1, C_2, and C_3 were collected, the solvent was evaporated in a rotating vacuum evaporator at 30 °C, and the residues were recrystallized from acetone. The crystals sharply melted at 122.9–123.5 °C (C_1), 86.3–86.8 °C (C_2), and 127.0–127.5 °C (C_3); the thermometer of the microscope was calibrated before use. The compounds C_1 and C_3 were obtained in amounts of 3–5 mg, whereas only 1 mg of compound C_2 was isolated.

Identification of Unknown Tryptamine Derivatives. Mass spectra were recorded for both the unknown compounds C_1, C_2, and C_3 and the reference compounds of C_{20}-5-HT (Ib) and C_{22}-5-HT (Ic). The results are shown in Table I. The spectra of Ib and Ic are in agreement with the literature (Wurziger and Harms, 1969; Hubert et al., 1977; Folstar et al., 1979). The mass spectrum of C_1 gave a molecular ion peak (M^+) at m/e 486 and fragmentation peaks at m/e 468 ($M^+ - H_2O$), 328 ($C_{19}H_{39}OCONH_3^+$), 311 ($C_{19}H_{39}OCO^+$), 310 ($C_{19}H_{39}OCONH_3^+ - H_2O$), 159 ($M^+ - C_{19}H_{39}OCONH_2$), and 146 ($M^+ - C_{19}H_{39}OCONHCH_2$). The fragmentation ions of C_1, attributed to the side chain, contain one more oxygen than the corresponding fragmentation ions of Ib. This is in agreement with the differences found between the molecular formulas of C_1 and Ib as calculated from the molecular ions. The mass spectra of C_2 and C_3 showed M^+ at m/e 514 and fragmentation peaks at m/e 496 ($M^+ - H_2O$), 356 ($C_{21}H_{43}OCONH_3^+$), 339

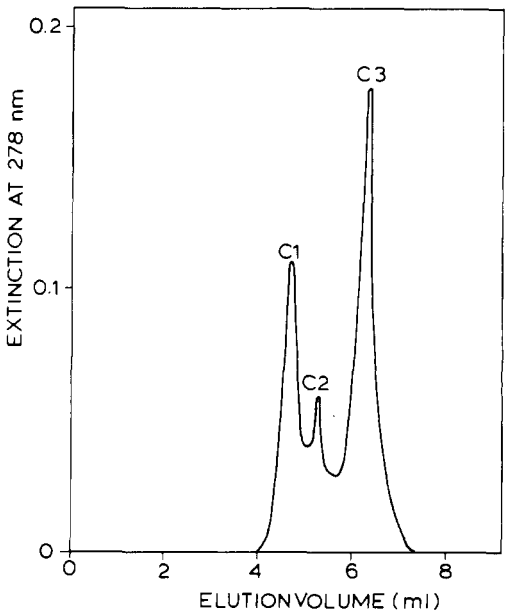

Figure 1. Fractionation of unknown tryptamine derivatives by high-pressure liquid chromatography on an analytical LiChrosorb 10RP18 column with methanol/acetonitrile (4:1, v/v) at 0.4 mL/min.

($C_{21}H_{43}OCO^+$), 338 ($C_{21}H_{43}OCONH_3^+ - H_2O$), 159 ($M^+ - C_{21}H_{43}OCONH_2$), and 146 ($M^+ - C_{21}H_{43}OCONHCH_2$). The mass spectra of C_2 and C_3 indicate that C_2 and C_3 are isomeric compounds and differ from Ic with respect to the presence of one more oxygen in the side chain. The IR spectra of C_1 and C_3 were almost identical with those of the reference compounds of Ib and Ic. For all spectra, major bands were found at 3500–3100 (NH, OH), 2960 and 2860 (CH), 1640 (C=O), 1590 (C–C aromatic ring), 1470, 1420, and 1370 (CH side chain), 1250–1170 (C–O aromatic ring), 850–790 (CH aromatic ring) and 720 (CH_2 side chain) cm^{-1}. For C_1 and C_3 another band is found at 1060 cm^{-1}, which indicates a primary alcohol; simultaneously an increase of the intensity of the OH band in the 3500–3100 cm^{-1} range was observed. Interpretation of the spectrum of C_2 at low frequencies was not possible as the amount of C_2 was not sufficient for recording a good spectrum.

The ^1H NMR spectra of C_1 and C_3 (in pyridine–carbon tetrachloride 2:3, v/v) indicated the absence of one CH_3 group that was found at δ 0.85 (t, J = 4 Hz) in the spectrum of Ic. The remaining peaks in the ^1H NMR spectra of C_1, C_3, and reference compound Ic were similar: δ 1.22 and 1.60 ($-(CH_2)_n-$ side chain), 2.12 (t, J = 7 Hz, $C{=}OCH_2$ side chain), 2.92 (t, J = 7 Hz; $C^\alpha H_2$ side chain), 3.58 (q, $J_{\alpha,\beta}$ = 7 Hz, $J_{\beta,NH}$ = 7 Hz, $C^\beta H_2$ side chain), 4.00 (OH, NH side chain), 6.61 (dd, $J_{6,7}$ = 8 Hz, $J_{6,4}$ = 2 Hz, C_6 aromatic ring), 6.68 (d, $J_{4,6}$ = 2 Hz, C_4 aromatic ring), 7.05 (d, $J_{2,NH}$ ≈ 1 Hz; C_2 aromatic ring), 7.15 (d, $J_{7,6}$ = 8 Hz; C_7 aromatic ring), 7.81 and 8.55 (OH, NH aromatic ring). Moreover the spectra of C_1 and C_3 show an increase of the intensity of the peak at δ 3.58 (m) due to a CH_2 group next to the primary OH group, having about the same chemical shift as CH_2 adjacent to the NH group in the side chain. The spectra downfield from δ 6.00 were recorded in Me_2SO. The assignment of the shifts was done on the basis of the literature (Daly and Witkop, 1967). The amount of C_2 was not sufficient for ^1H NMR. The UV spectra of C_1, C_2, and C_3 as well as those of reference compounds Ib, Ic and

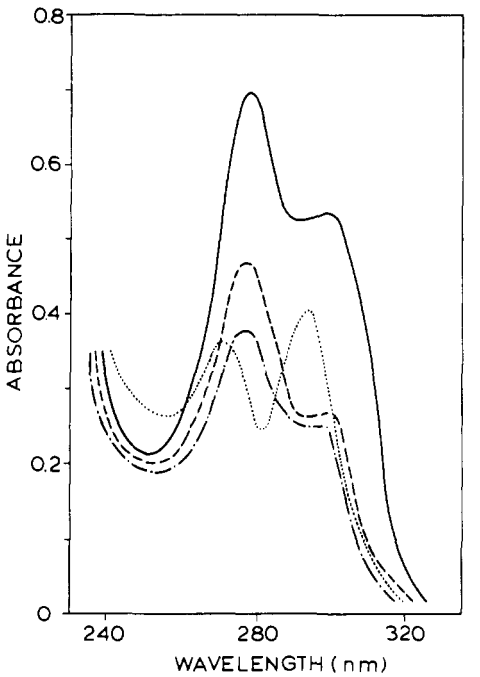

Figure 2. UV spectrum of C_{22}-5-HT (–), 5-hydroxytryptamine (- -), 6-hydroxytryptamine (···), and compound C_3 (–·–) in methanol.

5-hydroxytryptamine were found to be identical. In the literature it is known that the substitution pattern of the indole nucleus determines the shape of the UV spectrum (Marchelli et al., 1969); this is also illustrated by comparison of the UV spectra mentioned before and a spectrum of a reference compound of 6-hydroxytryptamine (Figure 2). Both the UV and the ^1H NMR spectra confirm the identity of C_1 and C_3 as 5-hydroxytryptamine derivatives. The absence of a CH_3 group in the side chain, shown by ^1H NMR, as well as the presence of a primary OH group, shown by IR, confirms a ω-hydroxyalkanoyl structure for the side chain of C_1 and C_3. Thus it can be concluded that compound C_1 is N_β-(20-hydroxyarachidoyl)-5-hydroxytryptamine (IIa) and compound C_3 is N_β-(22-hydroxybehenoyl)-5-hydroxytryptamine (IIb). All spectral data are consistent with these structures. The identity of compound C_2 could not fully be established; however enough evidence has been obtained that this minor constituent is a tryptamine derivative as well.

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