Triterpene Alcohols, 4-Methylsterols and 4-Desmethylsterols of Sal and Illipe Butters

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Four 4-desmethylsterols, four 4-methylsterols and eight triterpene alcohols were isolated from sal and illipe butters and identified by ^1H nuclear magnetic resonance and mass spectrometry. In addition to some components which had been shown to be present in these fats, several triterpene alcohols and one 4-methylsterol are described for the first time in these fats. An analytical method for detection of sal or illipe butters in foodstuffs is suggested.

We have determined the composition of 4-desmethylsterols, 4-methylsterols and triterpene alcohols extracted from the seed lipids of two plants belonging to the Dipterocarpaceae family, sal (*Shorea robusta*, G.) and illipe (*Shorea stenoptera*, B.). These plants grow in Indonesia and tropical Asia. The fats obtained from the seeds are used in the food and cosmetic industries, and can be detected by analysis of their triterpenic and steroidic components. Previous studies (1) report only frequently found compounds, and our aim was to confirm prior investigations and to look for other components. Because gas liquid chromatographic (GLC) determination of relative retention time (RRT) of compounds is not sufficient proof of structure, nonambiguous identification was performed by mass spectrometry (MS) and proton nuclear magnetic resonance (^1H NMR); the latter technique required pure components.

MATERIALS AND METHODS

Sal and illipe butters were of commercial origin (Aarhus Oil, Denmark). Saponification of 100 g of each fat with 1 L of a 1 M solution of alcoholic potash gave 1.6 g (1.6%) unsaponifiable material for sal fat and 1.4 g (1.4%) for illipe. Thirty g of neutral alumina (E. Merck, Darmstadt, Federal Republic of Germany) hydrated at 5% were used to chromatograph 1 g of the unsaponifiable fraction of each fat. The elution was carried out sequentially, first by hexane, and then by hexane/diethyl ether mixtures of increasing polarity. The triterpene alcohols (140 mg for both fats—14% of the unsaponifiable lipids), eluted by a 97:3 mixture (v/v), represented 0.22% of the total lipids for sal and 0.20% for illipe. The 4-methylsterols (16 mg or 0.026% of total sal fat and 12 mg or 0.017% for illipe) were eluted by a 96:4 mixture, and finally a 95:5 mixture separated the 4-desmethylsterols (300 mg or 0.46% for sal and 290 mg or 0.41% for illipe). A GLC chromatogram of each fraction was taken with a Delsi chromatograph equipped with a OV 1701 capillary column (25 m long and 0.32 mm i.d.), the vector gas was helium (1 bar pressure), the oven temperature was 270°C, and the injector and detector was 350°C. The RRT are expressed relative to cholesterol (retention time 12 min).

The triterpene alcohols (TA) were fractionated first by high performance liquid chromatography (HPLC) with a Waters chromatograph (Waters Associates, Milford, MA) equipped with a Merck Lichrosorb RP18 7 μ column, 25 cm long and 10 mm i.d.; detection by refraction index (RI) variation; and the mobile phase was anhydrous methanol at a flowrate of 4 mL/min. The purity of the fractions thus obtained was checked by GLC. The fractions corresponding to one main component were purified by analytical HPLC (same conditions as before, except with a Merck Lichrosorb RP18 5 μ column, 25 cm long and 4 mm i.d., flowrate of 1 mL/min). The fractions corresponding to mixtures of TA were separated by thin-layer chromatography (TLC) on 0.2 mm thick silica gel plates impregnated with silver nitrate (2,3). The elution was performed by several successive developments in CCl_4/CH_2Cl_2 (5:1, v/v). The bands were visualized under UV light at 350 nm after the plates had been sprayed with a 0.05% alcoholic solution of 2′,7′-dichlorofluorescein. The main bands were scraped off and extracted three times with hot chloroform. The resultant solutions were filtered, evaporated and the purity of each of the fractions thus obtained was checked by GLC. Three pure components [**1f, 4, 6**] were obtained by HPLC fractionation of sal fat triterpenic alcohols, and four others [**1e, 2e, 5, 7**] by TLC/$AgNO_3$ of the last fraction (four developments). In the same way, the illipe butter TA mixture gave three pure components by HPLC fractionation [**3, 4, 6**] and five others [**1e, 1f, 2e, 5, 7**] by TLC/$AgNO_3$ after six successive developments. Pure samples of 1–5 mg were obtained for each TA. A small peak (RRT = 1.48) appeared in the chromatogram of intact illipe TA that was not found in any isolated fraction. From its RRT, we presume it to be cycloartanol. It must be pointed out that the composition of the TA were nearly identical in the two fats, as confirmed by their RRT in GLC and HPLC, and also by NMR and MS spectra. In the two fats, the TA occurring in proportions of less than 2% were not isolated.

The 4-methylsterols (4-MS) were separated by semi-preparative HPLC, under the same conditions as above. Each fraction was purified by analytical HPLC (Merck Lichrosorb RP18 5 μ column, 25 cm long and 4 mm i.d., flowrate of solvent was 0.8 mL/min). Four identical 4-MS—**8e, 9e, 11e** and **11d**—were purified from each butter. 4-MS present in concentrations of less than 2% were not isolated.

In each fat, four identical 4-desmethylsterols (DS) were separated by semi-preparative HPLC and purified by analytical HPLC, giving pure **10a, 10b, 10c** and **10d.** All DS representing more than 1.0% of the total sterolic fraction were isolated.

The eight TA, the four MS and the four DS thus obtained were studied by ^1H NMR and by mass spectrometry (either pure or as TMS derivatives). The mass spectrometer was a Ribermag instrument (electron impact, 70 eV) (Delsi-Nermag, Argenteuil, France). The NMR instrument was a Bruker AC 360 (Bruker Spectrospin S.A., Wissembourg, France) and spectra were taken in $CDCl_3$ solution with TMS as the reference standard.

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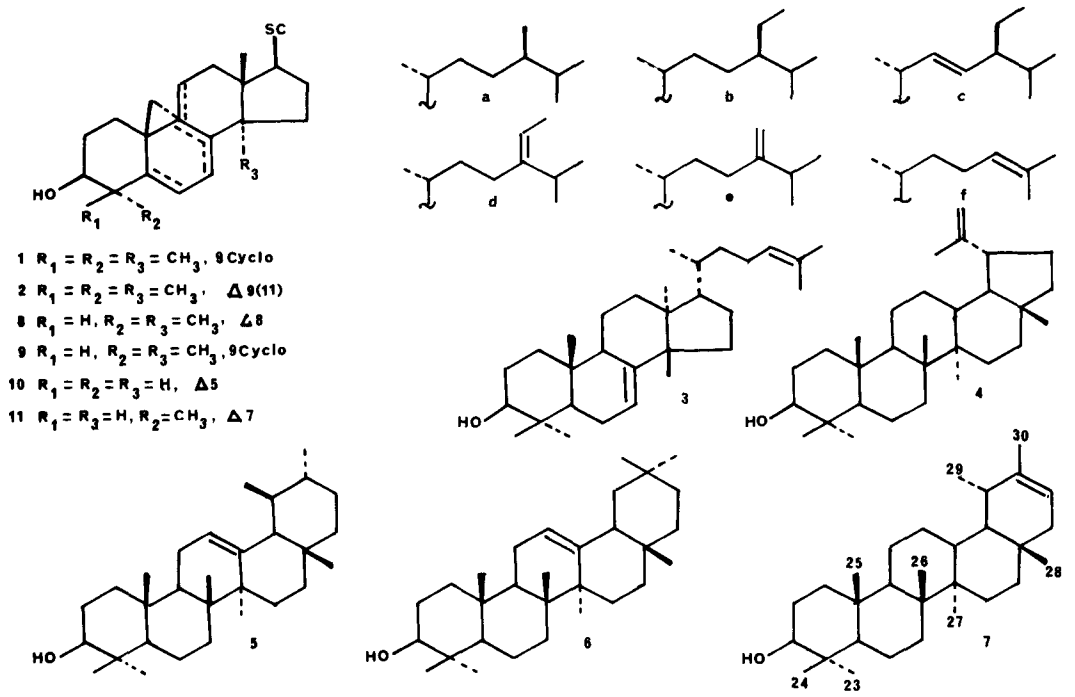

TABLE 1

Sal and Illipe Butters Triterpene Alcohols Data

Name	GLC RRT	Mass spectra of TMS derivatives and some diagnostic ions
Cycloartenol 1f (TMS)	1.63	498 (4), 483 (5), 408 (83), 393 (46), 365 (58), 339 (26), 297 (5), 286 (14), 255 (4), 241 (4)
24-Methylenecycloartanol 1e (TMS)	1.83	512 (2), 497 (3), 422 (38), 407 (19), 379 (31), 353 (8), 339 (2), 323 (2), 300 (12), 297 (8), 255 (3), 241 (3)
24-Methyleneparkeol 2e (free)	1.79	440 (22), 425 (100), 407 (38), 397 (21), 356 (8), 341 (11), 339 (4), 323 (8), 313 (88), 300 (6), 295 (10), 288 (5), 285 (6), 273 (22), 271 (8), 259 (18), 255 (11), 243 (13)
Butyrospermol 3 (free)	1.53	426 (20), 411 (100), 408 (5), 393 (31), 365 (2), 344 (2), 325 (3), 313 (8), 297 (4), 295 (3), 286 (4), 273 (7), 271 (9), 255 (9), 255 (7), 241 (8)
Lupeol 4 (TMS)	1.67	498 (17), 483 (5), 408 (7), 393 (7), 369 (14), 365 (2), 325 (3), 306 (3), 299 (4), 279 (10), 257 (6), 231 (16), 218 (37), 216 (9), 203 (36), 189 (56), 95 (100)
α-amyrin 5 (free)	1.67	426 (5), 411 (1), 218 (100), 207 (23), 203 (22), 189 (18)
β-amyrin 6 (TMS)	1.53	498 (6), 483 (1), 393 (1), 279 (5), 257 (2), 218 (100), 203 (38), 189 (20)
ψ-taraxasterol 7 (TMS)	1.97	498 (9), 483 (1), 408 (5), 393 (3), 369 (7), 365 (1), 326 (1), 279 (5), 257 (3), 231 (7), 218 (6), 203 (11), 189 (100)

TABLE 2

^{1}H NMR Data (360 MHz) of Sal and Illipe Butters Tetracyclic Triterpene Alcohols

Protons	Chemical shifts (δ ppm)			
	1f[a]	1e[b]	2e[c]	3[d]
CH_3 18 (*s*)	0.959	0.865	0.650	0.802
CH_3/CH_2 19	0.552/0.321	0.557/0.326	1.043	0.740
J Hz	4.3	4.0		
CH_3 21 (*d*)	0.877	0.892	0.905	0.845
J Hz	6.1	5.0	6.5	6.5
CH_3 26,27	1.677/1.598	1.027/1.021	1.027/1.022	1.678/1.599
J Hz		6.9	6.8	
CH_3 30 (*s*)	0.959	0.965	0.984	0.965
CH_3 31 (*s*)	0.802	0.807	0.816	0.854
CH_3 32 (*s*)	0.885	0.899	0.743	0.969
H 3 (dd)	3.280	3.281	3.210	3.235
J Hz	9.8/3.9	10.3/4.2	11.2/4.7	10.8/4.0

[a]H 24 (*t*): 5.094 (*J* = 6.3 Hz).
[b]H 28: 4.661/4.711.
[c]H 11 (*d*): 5.225 (*J* = 6.8 Hz), H 28: 4.712/4.658.
[d]H 7 (*d*): 5.248 (*J* = 4.0 Hz), H 24 (*t*): 5.089 (*J* = 6.7 Hz).

TABLE 3

^{1}H NMR Data (360 MHz) of Sal and Illipe Butters Pentacyclic Triterpene Alcohols

Protons	Chemical shifts (δ ppm)			
	4	5[a]	6[b]	7[c]
CH_3 23 (*s*)	0.962	0.999	0.940	0.976
CH_3 24 (*s*)	0.756	0.795	0.793	0.770
CH_3 25 (*s*)	0.826		0.971	0.855
CH_3 26 (*s*)	0.940	1.073	1.136	0.953
CH_3/CH_2 29	4.560/4.680	0.794 (*d*)	0.872	0.986 (*d*)
J (Hz)	2.2	5.8		7.2
CH_3 30	1.675	0.919 (*d*)	0.872	1.630
J (Hz)		4.0		
H 3 (dd)	3.179	3.224	3.221	3.205
J Hz	11.2/5.0	10.3/5.6	11.2/5.2	11.2/5.0

[a]H 12 (*t*): 5.130 (*J* = 3.6 Hz).
[b]H 12 (*t*): 5.184 (*J* = 3.6 Hz).
[c]H 21 (*d*): 5.260 (*J* = 7.2 Hz).

TABLE 4

Sal and Illipe Butters 4-Methylsterols Data

Name	GLC RRT	Mass spectra of TMS derivatives and some diagnostic ions
24-Methylenelophenol **11e**	1.51	484 (43%), 469 (6), 400 (86), 394 (12), 379 (13), 357 (100), 310 (25), 267 (11), 242 (18), 227 (21)
Citrostadienol **11d**	1.86	498 (8), 483 (6), 400 (86), 393 (4), 385 (5), 357 (100), 310 (6), 295 (8), 267 (13), 241 (9), 227 (11)
Obtusifoliol **8e**	1.37	426 (34), 411 (100), 408 (2), 393 (14), 383 (4) 365 (2), 327 (11), 309 (6), 327 (6), 285 (7), 259 (10), 245 (31), 241 (7), 227 (12)
Cycloeucalenol **9e**	1.54	426 (5), 411 (4), 408 (2), 393 (1), 326 (20), 285 (100), 241 (4), 227 (12)

RESULTS

Four of the TA (**1e, 1f, 2e, 3**) lost an eight or nine carbon atom side chain in MS, making them tetracyclic (Table 1), and the other four (**4** to **7**) were pentacyclic. Moreover, **1e** and **1f** gave a fragmentation (M − C_9H_{14} − TMSOH), which is typical of the cycloartane series (4). This was confirmed in NMR (Table 2) by the chemical shifts and the multiplicity of the two protons on C19. So, **1f** ($C_{30}H_{50}O$) is cycloartenol and **1e** ($C_{31}H_{52}O$) is 24-methylenecycloartanol because their spectroscopic data are identical to those given by the literature for these TA (5,6). **2e** ($C_{31}H_{52}O$) and **3** ($C_{30}H_{50}O$) have a monounsaturated tetracyclic nucleus. Chemical shifts of ethylenic protons and methyl groups proved that **2e** was 24-methyleneparkeol (7) and **3** was butyrospermol (8). The *R* configuration of C20 in **3** was determined by the precise chemical shift of Me21 (8–10). MS data (11,12) and RRT in GLC (13) were in good agreement with these structures.

In MS, the base peak of **5** and **6** was m/e 218. This fragment corresponds to retro Diels-Alder cleavage of ring C in a 12–13 unsaturated pentacyclic TA (14,15). The eight methyl groups of **5** appeared in NMR (Table 3) as six singlets and two doublets, whose chemical shifts corresponded to those of α-amyrin. The eight methyl singlets of **6** allowed an easy identification as β-amyrin. Owing to its seven methyl singlets, one of which at a relatively low field, and the typical pattern of its two geminate ethylenic protons, **4** was identified as lupeol. It must be pointed out that both **4** and **5** had almost the same RRT in GLC.

The MS base peak of **7** at m/e 189 implied a double bond in rings D or E. No other fragmentation was useful for identification. The NMR spectrum showed one intracyclic ethylenic proton, one doublet and seven singlets, one of which, at low field, corresponded to methyl groups. These data, coupled with the high value of its RRT in GLC (13), suggested ψ-taraxasterol. Its NMR spectrum was

TABLE 5

^{1}H NMR Data (360 MHz) of Sal and Illipe Butters 4α-Methylsterols

		Chemical shifts (δ ppm)		
Protons	8	9	11d[a]	11e
CH_3 18 (*s*)	0.714	0.973	0.534	0.540
CH_3/CH_2 19	0.971	0.141/0.391	0.828	0.829
J Hz		3.8		
CH_3 21 (*d*)	0.930	0.900	0.951	0.954
J Hz	6.5	6.5	6.5	6.5
CH_3 26,27	1.027/1.024	1.031/1.027	0.978	1.029/1.024
J Hz	6.8	6.8	7.2	6.8
CH_3 30 (*d*)	0.996	0.982	0.988	0.989
J Hz	6.1	6.1	6.5	6.1
CH_3 32 (*s*)	0.889	0.897	—	—
H 3 (*dt*)	3.099	3.216	3.121	3.118
J Hz	10.6/4.7	10.1/4.3	10.9/4.3	10.8/4.2
H 7 (*d*)			5.182	5.182
J Hz	—	—	5.8	5.8
H 28	4.663/4.716	4.663/4.717	5.109	4.659/4.714

[a]CH_3 29 (*d*): 1.590 (*J* = 6.8 Hz).

TABLE 6

Sal and Illipe Butters 4-Desmethylsterols Data

Name	GLC RRT[a]	Mass spectra of TMS derivatives and some diagnostic ions
Campesterol **10a**	1,24	472 (16), 457 (9), 382 (39), 367 (20), 343 (58), 261 (12), 255 (17), 227 (6), 213 (13), 129 (100)
Stigmasterol **10c**	1,32	484 (23), 394 (31), 379 (13), 355 (12), 351 (18), 283 (7), 255 (43), 253 (12), 228 (9), 213 (12), 129 (100)
Sitosterol **10b**	1,53	486 (13), 471 (7), 396 (30), 381 (14), 357 (12), 255 (10), 228 (2), 213 (12), 129 (100)
Isofucosterol **10d**	1,57	484 (8), 469 (6), 394 (10), 386 (84), 371 (11), 355 (10), 296 (65), 281 (45), 255 (11), 253 (10), 228 (7), 213 (13), 129 (100)

[a]Relative to cholesterol.

recently published (16), and that data is in good agreement with ours (Table 3).

The four 4-MS have been found frequently. They were identified as cycloeucalenol (**9e**), obtusifoliol (**8e**), 24-methylenelophenol or gramisterol (**11e**) and citrostadienol (**11d**). Their MS and NMR data (Tables 4 and 5) and their RRT in GLC were identical to those reported in the literature (5,17–19) for these compounds.

The four DS also corresponded to the commonly found compounds campesterol (**10a**), sitosterol (**10b**), stigmasterol (**10c**) and isofucosterol (**10d**) (Tables 6 and 7). They were identified by MS (5,20), NMR (5,21) and RRT data (13), taking care of configurations 24*R* for **10a** and **10b**, 24*S* for **10c** and 24(28)*Z* for **10d** (5).

If we consider all the triterpenic components (Table 8), we can see a large predominance of desmethylsterols and a very low proportion of 4-methylsterols in the two fats.

Among the nine identified TA, five (α and β-amyrin, butyrospermol, lupeol, ψ-taraxasterol) come from a chair/chair/chair/boat cyclization of 2,3-epoxysqualene. They represent an unusually important part of the total TA fraction, 63% in sal and 75% in illipe. The other four result from a chair/boat/chair/boat cyclization. With the exception of 24-methyleneparkeol, they are the precursors of the 4-MS and MS which play an important role in the structure of plasmic cellular membranes.

Identification of fats and searches for mixtures or adulterations is often performed by GLC analysis of the desmethylsterols fraction. Because these components are often similar in many fats, we believe that the GLC analysis of triterpenic alcohols would give better results. In the case of sal and illipe butters, which are used along with others in the food industry, the detection of ψ-taraxasterol would be an important indication of their presence. Indeed, this rarely found component can be easily identified by its high RRT.

This study gives a better knowledge of triterpenic composition of sal and illipe fats. These two plants belong to the same botanical family and their constituents (with one exception) are the same, but the proportions differ slightly. The desmethylsterols and 4-methylsterols fractions contained components that had been described in earlier literature (1). We found seven compounds in sal and nine in illipe among the triterpenic alcohols. Two of them, 24-methylene parkeol and ψ-taraxasterol, had not been previously described in these fats. The latter is especially interesting, because it is rarely found in plant lipids, and is easy to detect.

TABLE 7

^{1}H NMR Data (360 MHz) of Sal and Illipe Butters 4-Desmethylsterols

Protons	Chemical shifts (δ ppm)			
	10a	**10b**	**10c**[a]	**10d**
CH_3 18 (*s*)	0.664	0.681	0.700	0.682
CH_3 19 (*s*)	0.991	1.009	1.012	1.000
CH_3 21 (*d*)	0.894	0.922	1.025	0.935
J Hz	6.3	6.3	6.3	6.3
CH_3 26 (*d*)	0.834	0.834	0.855	0.697
J Hz	6.3	7.2	6.3	6.3
CH_3 27 (*d*)	0.786	0.814	0.805	0.697
J Hz	6.3	7.2	6.3	6.3
H/CH_3 28	0.757	—	—	5.090
J Hz	5.4 (*d*)	—	—	5.4 (*q*)
CH_3 29	—	0.846	0.806	1.585
J Hz		7.9 (*t*)	6.8 (*t*)	5.4 (*d*)
H 3 (*tt*)	3.504	3.523	3.520	3.512
J Hz	10.8	10.8	10.8	10.8
	5.4	5.4	5.4	5.4
H 6 (*d*)	5.335	5.350	5.346	5.340
J Hz	5.4	5.4	5.4	5.4

[a]H 22,23: 5.020/5.152 (*dd*, *J* = 14.2/9.0 Hz).

TABLE 8

Percent Composition of Sterols and Triterpene Alcohols of Sal and Illipe Fats

	Sal	Illipe
Triterpene alcohols	30.4%	31.5
β-amyrin	9.2	3.1
Butyrospermol	—	2.2
Cycloartenol	7.1	1.1
α-amyrin + lupeol	7.3	17.4
24-Methyleneparkeol	1.2	0.9
24-Methylenecycloartanol	2.7	4.1
ψ-Taraxasterol	2.7	0.9
Cycloartanol	—	0.8
Unknown	0.2	1.0
4α-Methylsterols	4.3	3.2
Obtusifoliol	0.7	0.5
24-Methylenelophenol	1.0	0.9
Cycloeucalenol	1.4	0.6
Citrostadienol	0.9	0.8
Unknown	0.3	0.4
4-Desmethylsterols	65.3	65.3
Campesterol	14.8	12.7
Stigmasterol	5.6	4.2
Sitosterol	40.5	44.2
Isofucosterol	3.2	3.6
Unknown	1.2	0.6

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