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SEPARATION OF 24-METHYLENECYCLOARTANOL FROM CYCLOARTENOL VIA A CHEMICAL METHOD

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ABSTRACT.—A simple chemical method for the separation of 24-methylenecycloartanol [**2**] from a mixture of cycloartenol [**1**] and **2** has been developed. The method consists of five reaction steps: converting the 3-OH into 3-*O*-tetrahydropyran (3-OTHP), hydroboration of the double bond, mesylation, regeneration of the double bond, and removal of the protecting group. This approach is practical for large scale separation.

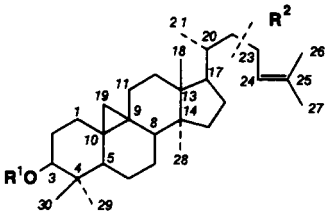
Cycloartenol [**1**] and 24-methylenecycloartanol [**2**] are two major triterpene alcohols present as ferulate esters in rice bran oil (1). Because of structural resemblance, these two compounds are difficult to separate via conventional tlc or cc. Due to their abundance and unique structures, both compounds having a cyclopropyl moiety at C-9, C-10, and C-19, they are interesting substrates for microbial transformation. Thus, separation of these two compounds is often an obligatory preliminary task. Several attempts to separate these two materials, without modification of the structures, via Si gel columns were unsuccessful. We have developed the following method for separating **2** from mixtures of **1** and **2**.

RESULTS AND DISCUSSION

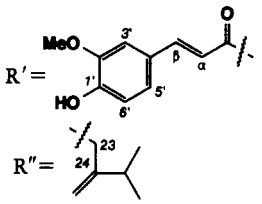
The key strategy for separating **2** from **1** was to convert them into separable compounds, then regenerate **2** after separation of the derivatives. Compound **1** contains a trisubstituted double bond while **2** contains an exomethylene group. Upon hydroboration of the mixture, a secondary alcohol was produced from **1** and a primary alcohol was produced from **2**. The polarity difference between these two alcohols allows their separation.

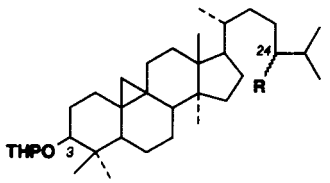
Mixtures of ferulate esters of **1** and **2** are obtained directly via recrystallization ($MeOH/CHCl_3$) of oryzanol, an ester mixture of triterpenes and steroids from rice brans (1). The ferulates show the α,β-trans protons at δ 6.27 and 7.58 (J_{trans} = 15.9 Hz), C-19 protons at δ 0.59 and 0.34 (J_{gem} = 4.3 Hz), C-24 protons of **1** at δ 5.09 (t, J = 6.6 Hz), C-31 protons of **2** at δ 4.70 (d, J = 2.6 Hz), and characteristic carbon signals of the cyclopropyl group at δ 20.1 (s, C-9), 26.2 (s, C-10), and 29.8 (t, C-19). This mixture, after basic hydrolysis (3 N KOH/EtOH, reflux), yielded a 1:1 mixture of **1** and **2** calculated from the integration of the olefinic proton signals. Without further separation, the alcohol fraction was protected with THP (2). After partial purification on a Si gel column, hydroboration (diborane/THF, H_2O_2, NaOH) (3) was performed on the mixture. The reaction gave two products, **5** and **6**, which were separable by tlc and by Si gel cc.

The secondary alcohol **5** derived from **1**-THP shows hydroxyl absorption at 3450 cm^{-1} and a fragment ion peak **A** at *m/z* 444 and the base peak **B** at *m/z* 85 in the eims. The ^{13}C-nmr spectrum shows an additional oxygenated methine signal at δ 77.0, in addition to C-3 (δ 87.8) and C-1′ (δ 101.8) signals, which also support its structure. The primary alcohol **6** obtained from **2**-THP shows a hydroxy absorption at 3490 cm^{-1}. Its eims spectrum shows the base peak **B** at *m/z* 85 supporting the THP moiety, and a fragment ion **C** at *m/z* 458. In the ^{1}H-nmr spectrum of **6**, the signals of the hydroxymethylene protons appear at δ 3.55 (br d, J = 3.2 Hz), and the protons at C-19 of the cyclopropyl ring appear at δ 0.54 and 0.29 as an AB spin system (J = 4.1 Hz). The

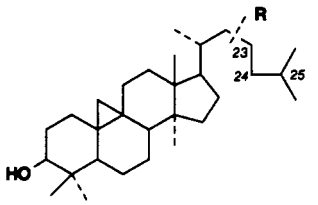


1 $R^1=H$
1 ferulate $R^1=R'$
2 $R^1=H$, $R^2=R''$
2 ferulate $R^1=R'$, $R^2=R''$
3 $R^1=THP$
4 $R^1=THP$, $R^2=R''$





5 R=OH
6 $R=CH_2OH$
7 R=OMs
8 $R=CH_2OMs$



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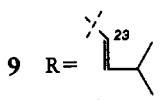
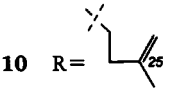
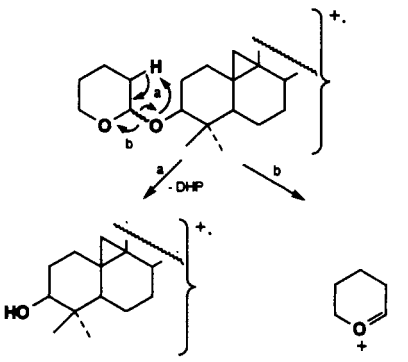




^{13}C-nmr spectrum shows additional oxygenated methylene signals at δ 63.5 and δ 62.6 (C-5′ of THP). These data confirm the structure of **6**.

Treatment of **5** and **6** with mesyl chloride in pyridine (4) yielded mesylates **7** and **8**, respectively. The ^{1}H-nmr spectra of **7** and **8** both show the characteristic methyl signal



A *m/z* 444 (from **5**) **B** *m/z* 85
C *m/z* 458 (from **6**)

of the mesyl group at δ 2.97. The β-elimination of the mesyl group of **8** with basic alumina in refluxing $CHCl_3$ (5), followed by removal of the THP protecting group with HOAc-THF-H_2O (4:2:1) (2), gave **2** as the sole product in 65% yield. This product was identical (mp, ms, nmr) with reported data for 24-methylenecycloartanol (6). The same treatment of **7** gave a mixture containing **1** as the major component accompanied by two minor inseparable byproducts, **9** and **10**, in a ratio of 10:2:1 from integration of the corresponding olefinic proton signals at δ 5.08 (br t, $J = 6.6$ Hz, **1**), 5.34 (m, **9**), and 4.65 (br s, **10**). The structures of **9** and **10** were tentatively proposed and supported by a ^{13}C-nmr spectrum of the mixture which showed three sets of olefinic carbons each composed of two carbon signals, the major peaks being at δ 130.7 (s, C-25, **1**) and 125.3 (d, C-24, **1**). The other two sets of signals appeared at δ 138.3 (d, C-24, **9**), 125.9 (d, C-23, **9**), 146.2 (s, C-25, **10**), and 109.5 (t, C-26, **10**). Hydrogenation of the mixture led to compound **11** as the sole product. The ^{13}C-nmr spectrum of **11** was almost superimposable with the carbon chemical shifts of the ring skeleton of cycloart-24-ene-3β,26-diol (7) and with the side chain of cholestan-3β-ol (8). Compound **11** was thus identified as cycloartanol, and these results support the suggested structures of **9** and **10**.

Formation of **9** may be rationalized by nonregiospecific elimination of the mesyl group which produced either Δ^{23} as in **9** or Δ^{24} as in **1**. Compound **10**, however, could be obtained via delocalization of the carbonium ion from C-24 to C-25 which, after deprotonation, yielded either **1** (Δ^{24}) or **10** (Δ^{25}).

This facile approach can be scaled up and is practical to separate 24-methylenecycloartanol [**2**] from oryzanol. Although pure cycloartenol [**1**] has not been separated from the mixture of **9** and **10**, cycloartanol [**11**], the product after hydrogenation, is also an interesting substrate for microbial transformation studies.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Oryzanol was supplied by Dr. Hsien-Jer Chen, Food Industry Research and Development Institute, Hsinchu, Taiwan. Optical rotations were measured on a JASCO DIP-181 Digital Polarimeter. Ir spectra were recorded on a Perkin-Elmer 1760-X Infrared FT Spectrometer. Nmr spectra were recorded on a Bruker AC-80 or AM-300 instrument using the solvent peak as reference standard (in $CDCl_3$, δ 7.24 ppm for ^{1}H nmr and 77.0 ppm for ^{13}C nmr). Mass spectra were taken on a Finnigan Mat 4500 series GC/MS spectrometer.

MIXTURE OF CYCLOARTENOL [**1**] AND 24-METHYLENECYCLOARTANOL [**2**].—To oryzanol (40.0 g) dissolved in a minimum of warm $CHCl_3$ was added MeOH dropwise till turbidity occurred. The solution was kept at ambient temperature, and the resulting crystals were filtered. This process was repeated

twice to yield 7 g of colorless crystals, the ferulates of **1** and **2**. The crystals were refluxed in 2.5 N alcoholic KOH (600 ml) for 3 h. The cooled reaction mixture was diluted with H_2O (600 ml), acidified with concentrated HCl to pH 2, and extracted with $CHCl_3$ (400 ml × 3). The $CHCl_3$ layer was washed with 8% $NaHCO_3$ (300 ml × 3) to remove ferulic acid and dried over Na_2SO_4, and the solvent was removed to give 4.8 g of a mixture containing **1** and **2** (99% yield).

PREPARATION OF **3** (**1**-THP) AND **4** (**2**-THP).—The mixture of **1** and **2** (4.0 g, about 9.24 mmol) dissolved in CH_2Cl_2 (55 ml) was mixed with dihydropyran (DHP, 2.2 ml) and *p*-TsOH (125 mg) (2). The reaction mixture was stirred at room temperature for 1 h, then diluted with an equal volume of CH_2Cl_2, washed with 1% KOH (120 ml) and H_2O (60 ml × 2) to remove *p*-TsOH, dried over Na_2SO_4 and evaporated to a yellowish viscous residue. The residue was chromatographed on a Si gel column (150.0 g, 70–230 mesh) eluted with 2% EtOAc in hexane to give a mixture of **3** (**1**-THP) and **4** (**2**-THP) (4.5 g, 94% yield).

PREPARATION OF **5** AND **6**.—To the THP analogues of **1** and **2** (4.5 g, about 8.70 mmol), dissolved in freshly distilled THF (50 ml) in a 100-ml round-bottomed flask, was slowly added 1.0 M B_2H_6 in THF (7.5 ml) in an ice bath (3). The reaction mixture was stirred for 1 h, and 3 N NaOH (4 ml) and 30% H_2O_2 (2.5 ml) were added. The reaction was continued for another 1 h at 30–50° with stirring. The reaction mixture was then diluted with H_2O (90 ml) and extracted with Et_2O (100 ml × 3). The Et_2O layer was dried over Na_2SO_4 and evaporated to give 4.2 g of residue which showed two spots by tlc [Si gel, R_f 0.41, **5**: R_f 0.35, **6**; solvent system Me_2CO-hexane (1:4)]. These two spots were separated via a Si gel column (160 g) eluted with 1–2% of Me_2CO in hexane to yield 1.56 g of **5** and 1.67 g of **6** (70% total yield).

3-O-Tetrahydropyranyl-24-hydroxycycloartanol [**5**].—Mp 124–126°; ir (KBr, cm^{-1}) ν max 3450, 3040, 2950 (s), 2875 (s), 1475, 1460, 1455, 1385, 1370; eims *m/z* (rel. int.) $[M-DHP]^+$ 444 (5), ($[M-DHP-H_2O]^+$ 426, 10), 411 (5), 383 (6), 304 (10), 203 (10), 175 (14), 135 (12), 121 (15), 107 (18), 95 (25), 85 (C, 100); ^{13}C nmr ($CDCl_3$) δ 101.9 (d, C-2′ in THP), 87.8 (d, C-3), 77.0 (d, C-24), 62.6 (t, C-6′ in THP); ^1H nmr ($CDCl_3$) δ 0.29 and 0.54 (H-19, AB q, J = 4.1 Hz), 4.64 (m, H-1′ in THP), 3.92 (m, H-3), 3.50–2.99 (m, 3 × H, H-6′ in THP and H-24).

3-O-Tetrahydropyranyl-24-hydroxymethylcycloartanol [**6**].—Mp 141–142°; ir (KBr, cm^{-1}) ν max 3490 (m), 3040 (w), 2950 (s), 2875 (s), 1475 (m), 1462 (br m), 1445 (w), 1385 (m), 1370 (br m); eims *m/z* (rel. int.) $[M-DHP]^+$ 458 (5), 441 (6), 425 (7), 318 (10), 203 (8), 175 (15), 135 (10), 121 (12), 107 (14), 95 (35), 85 (C, 100); ^1H nmr ($CDCl_3$) δ 0.29 and 0.54 (H-19, AB q, J = 4.1 Hz), 4.64 (m, H-1′ in THP), 3.93 (m, H-3), 3.55 (br d, J = 3.2 Hz, H-31), 3.57–3.00 (m, 2× H, H-6′ in THP); ^{13}C nmr ($CDCl_3$) δ 101.8 (d, C-2′ in THP), 87.8 (d, C-3), 63.8 (t, 24-*C*H_2OH), 62.6 (t, C-6′ in THP).

PREPARATION OF CYCLOARTENOL [**1**] AND 24-METHYLENECYCLOARTANOL [**2**].—*Mesylation*.—To **5** (160 mg, 0.30 mmol) dissolved in pyridine (2.0 ml) was added mesyl chloride (0.3 ml) dropwise in an ice bath (4). The reaction mixture was stirred at 0° for 30 min and then at ambient temperature for 1 h. To the reaction solution was then added pyridine-H_2O (2:1) (0.15 ml). After stirring in an ice bath for 15 min, H_2O (100 ml) was added to the mixture and it was extracted with Et_2O (100 ml × 3). The Et_2O layer was dried over $MgSO_4$ and evaporated to give 204 mg of residue, which was chromatographed on a Si gel column (10 g) eluted with 2–4% Me_2CO in hexane to give mesylate **7** (180 mg, 98% yield): ir ν max (KBr, cm^{-1}), 3040 (w), 2940 (s), 2860, 1470, 1355 (s), 1175 (s), 1115, 1022 (s), 976 (s); ^1H nmr δ ($CDCl_3$) 2.97 (s, -OSO_2*Me*), 0.54 and 0.31 (AB q, J = 4.1 Hz, H-19).

Similarly, compound **6** (160.0 mg, 0.295 mmol) was converted into mesylate **8** (173.4 mg, 95% yield): ir ν max (KBr, cm^{-1}) 3040 (w), 2940 (s), 2870, 1445, 1378, 1355 (s), 1340 (br s), 1200 (m); ^1H nmr δ ($CDCl_3$) 2.98 (s, -OSO_2*Me*), 0.55 and 0.32 (AB q, J = 4.2 Hz, H-19).

β-*Elimination and deprotection*.—Compound **8** (173.4 mg, 0.28 mmol) dissolved in $CHCl_3$ (8 ml) was mixed with Al_2O_3 (basic, 5.0 g) (5). The mixture was stirred under reflux for 2.5 h, and Al_2O_3 was removed by filtering. The alumina was washed with $CHCl_3$. The combined $CHCl_3$ filtrate was evaporated and the residue was mixed with HOAc-THF-H_2O (4:2:1) (6 ml) (2). The resulting suspension was stirred at 45° for 4 h, and the solvent was evaporated. The residue was purified via a Si gel column (12 g, 230–400 mesh) eluted with 2–3% EtOAc in hexane to give **2** as the sole product (80 mg, 65% yield from **8**).

In a similar manner, mesylate **7** (180 mg, 0.297 mmol) was converted into a mixture containing **1** and two minor products, **9** and **10** (90 mg, 71% yield).

24-Methylenecycloartanol [**2**].—Mp 121° [lit. (6) 118–120°]; ir (KBr, cm^{-1}) ν max 3440 (m), 3090 (w), 3050 (w), 2970 (s), 2940 (s), 2875 (s), 1645 (m), 1470 (br m), 1380 (m), 1100 (m), 1050 (m), 1020 (m), 1000 (m), 890 (s, $=CH_2$); eims *m/z* (rel. int.) $[M]^+$ 440 (calcd for $C_{31}H_{52}O$) (27), $[M-Me]^+$ 425 (30), 422 (58), 407 (38), 379 (22), 300 (56), 216 (38), 203 (49), 201 (27); ^1H nmr ($CDCl_3$) δ 4.67 (br d, J = 2.6 Hz, H-31), 0.54 and 0.30 (AB q, J = 4.1 Hz, H-19); ^{13}C nmr ($CDCl_3$) δ 32.0 (t, C-1), 30.5 (t, C-2), 78.8 (d, C-3), 40.5 (s, C-4), 47.2 (d, C-5), 21.1 (t, C-6), 28.1 (t, C-7), 47.9 (d, C-8), 20.1 (s, C-9),

26.2 (s, C-10), 26.0 (t, C-11), 35.6 (t, C-12), 45.4 (s, C-13), 48.9 (s, C-14), 33.0 (t, C-15), 26.6 (t, C-16), 52.3 (d, C-17), 18.0 (q, C-18), 29.8 (t, C-19), 36.1 (d, C-20), 18.4 (q, C-21), 31.4 (t, C-22), 28.1 (t, C-23), 156.8 (s, C-24), 36.1 (d, C-25), 21.9 (q, C-26), 22.0 (q, C-27), 19.3 (C-28), 25.5 (q, C-29), 14.0 (q, C-30), 106.0 (t, C-31) (7,8).

Cycloartanol [**1**].—The mixture of **1**, **9**, and **10** (9.5 mg) was catalytically hydrogenated over Pd/C (5%, 10 mg) under H_2 (1 atm) at 25° overnight. The suspension, after filtering through a celite pad, was evaporated to give 9.3 mg of residue. The product **11**, recrystallized from MeOH, gave the following: mp 66–67°; ir (KBr, cm^{-1}) ν max 3340 (m), 3035 (w), 2950 (s), 2925 (s), 2860 (s), 1465 (m), 1460 (m), 1450 (m), 1440 (m), 1380 (m), 1370 (m), 1095 (m), 1043 (m), 1020 (m), 1003 (m), 992 (m); eims *m/z* (rel. int.) $[M]^+$ 428 (calcd for $C_{30}H_{52}O$) (2), 429 (0.3), 413 (3), 410 (3), 395 (5), 367 (3), 315 (2), 175 (16), 135 (18), 121 (25), 107 (31), 95 (60), 71 (27), 57 (51), 43 (100); ^1H nmr ($CDCl_3$) δ 3.26 (dd, J = 9.6, 5.2 Hz, H-3), 0.55 and 0.31 (AB q, J = 4.4 Hz, H-19); ^{13}C nmr ($CDCl_3$) δ 32.1 (t, C-1), 30.5 (t, C-2), 78.9 (d, C-3), 40.5 (s, C-4), 47.2 (d, C-5), 21.1 (t, C-6), 28.2 (t, C-7), 47.9 (d, C-8), 20.1 (s, C-9), 26.0 (s, C-10), 26.0 (t, C-11), 35.6 (t, C-12), 45.4 (s, C-13), 48.8 (s, C-14), 33.0 (t, C-15), 26.6 (t, C-16), 52.5 (d, C-17), 18.0 (q, C-18), 29.9 (t, C-19), 36.1 (d, C-20), 18.4 (q, C-21), 36.5 (t, C-22), 24.1 (t, C-23), 39.6 (t, C-24), 28.0 (d, C-25), 22.4 (q, C-26), 22.8 (q, C-27), 19.4 (C-28), 25.5 (q, C-29), 14.0 (q, C-30).

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