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MANIPULATION BY 25-AZACYCLOARTANOL OF THE RELATIVE PERCENTAGE OF C_{10}, C_9 AND C_8 SIDE-CHAIN STEROLS IN SUSPENSION CULTURES OF BRAMBLE CELLS

PAULETTE SCHMITT,* ACHARAN S. NARULA,† PIERRE BENVENISTE* and ALAIN RAHIER*

* Laboratoire de Biochimie Végétale, E.R.A. N° 487 du C.N.R.S., Institut de Botanique, 28, rue Goethe, 67083-Strasbourg, Cedex, France; † Research School of Chemistry, The Australian National University, Canberra, A.C.T. 2600, Australia

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Key Word Index—*Rubus fruticosus*; Rosaceae; sterol biosynthesis; inhibitors of side chain C-24 and C-28 alkylation; 25-azacycloartanol.

Abstract – The addition of 25-azacycloartanol to the medium of suspension cultures of bramble cells resulted, after 6 weeks of growth, in a large decrease in the percentage of C_{10} side-chain sterols, sitosterol and isofucosterol (83% of the total in the control, 9% in the treated cells), and in a spectacular increase in the percentage of C_8 side-chain sterols, cycloartenol, desmosterol and cholesterol (less than 1% in the control, 53% in the treated cells). In addition the relative percentage of C_9 side-chain sterols, mainly 24-methylene cholesterol increased significantly (from 16 to 37%). A secondary effect of 25-azacycloartanol consisted in an increase of the percentage of Δ^{24} sterols and in a decrease of the percentage of sterols with a saturated side chain. These results are in agreement with an inhibition by 25-azacycloartanol of the C-24 and C-28 methyltransferases and of the Δ^{24} reductase.

INTRODUCTION

Higher plant sterols are generally a mixture of 24-alkylated sterols, 24-ethyl sterols generally accounting for more than 70% of the total sterols and 24-methyl sterols less than 30% of the total [1]. Sterols non-methylated at C-24 such as cholesterol are generally present in trace amounts. One can speculate at the ubiquitous presence of 24-alkylated sterols in higher plants. As sterols are constituents of membranes [2] and especially of the plasma membranes [3], it may be expected that the presence of 24-ethyl sterols in plant plasma membranes [4–6] could be correlated to some specific feature of the lipid composition and organization of the plant membranes. In order to test the likelihood of this hypothesis we planned to modify the relative percentage of 24-desmethyl, 24-methyl and 24-ethyl sterols in plants and to study the influence of this modification on the structure and function of membranes. Such a result was achieved by feeding plants with specific inhibitors of the *S*-adenosyl-L-methionine (SAM)-C-methyltransferases responsible for the introduction of the two extra carbon atoms at position C-24 of higher plant sterol. These C-methyltransferases involved in plant sterol biosynthesis have been studied previously [7–9]. Experiments have shown that some plant cell-free extracts when incubated in the presence of SAM-Me-^{14}C were capable of introducing one methyl group at C-24 in various Δ^{24} sterols [7–9]. These studies have also established that cycloartenol was the preferred Δ^{24} substrate suggesting that *in vivo* C-24-methylation occurs mainly at the cycloartenol level [8–9]. It has been found recently [9] that bramble cell free extracts can transfer a second methyl group to position C-28 of a suitable sterol possessing a methylene group at C-24. Of the substrates assayed, only 24-methylene lophenol was methylated, giving 24-ethylidene lophenol, a ubiquitous 4α-methyl sterol of plants. This finding suggests that 24-methylene lophenol could be the target of C-28 methylation *in vivo*. Analogous studies performed with yeast cell free extracts have shown that zymosterol was the preferred substrate for C-24-methylation in this material [10]. Accordingly, Avruch *et al.* [11] have grown yeast cells in the presence of 25-azadihydrozymosterol; this resulted in the accumulation of C_8 side-chain sterols such as cholesta-5,7,24-trien-3β-ol instead of ergosterol and showed that the drug inhibited strongly the SAM-zymosterol-C-24-methyltransferase. As cycloartenol is the preferred substrate of the C-24 methyltransferase in higher plant cells, we have synthesized 25-azacycloartanol (**1**) and studied its action on a cell free extract from maize embryos containing a microsomal C-24 methyltransferase [9, 12]. The results obtained in this *in vitro* study indicated that **1** was a potent

Nomenclature: Cycloartenol = 4,4,14α-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol (**2**); 24-methylenecycloartanol = 4,4,-14α-trimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (**3**); cyclo-eucalenol = 4α,14α,14α-dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (**4**); obtusifoliol = 4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol (**5**); 31-norcycloartenol = 4α,14α-dimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol (**6**); 31-norlanosterol = 4α,-14α-dimethyl-5α-cholesta-8,24-dien-3β-ol (**7**); 24-methylene-lophenol = 4α-methyl-5α-ergosta-7,24(28)-dien-3β-ol (**8**); 24-ethylidenelophenol = 4α-methyl-5α-stigmasta-7 *Z*-24(28)-dien-3β-ol (**9**); isofucosterol = stigmasta-5, *Z*-24(28)-dien-3β-ol (**10**); sitosterol = (24*R*)-24-ethyl-cholest-5-en-3β-ol (**11**); 24-methyl-enecholesterol = ergosta-5,24(28)-dien-3β-ol (**12**); camp-esterol = (24*R*)-24-methyl-cholest-5-en-3β-ol (**13**); desmosterol = cholesta-5,24-dien-3β-ol (**16**); cholesterol = cholest-5-en-3β-ol (**17**); β-amyrin = olean-12-en-3β-ol; α-amyrin = urs-12-en-3β-ol; 25-azacycloartanol = *N*,*N*-dimethyl-24-amino-25,26,27-tris-nor-4,4,14α-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol (**1**).

inhibitor of the SAM-sterol-C-24 and C-28 methyl transferases [12]. In the present paper we give results on the action of **1** on suspension cultures of bramble cells. The results show that the relative percentage of 24-desmethyl, 24-methyl and 24-ethyl sterols is dramatically changed following this treatment.

RESULTS

Synthesis of 25-azacycloartanol

25-Azacycloartanol (**1**) was synthesized by a method derived from that used in the synthesis of 25-azadihydrozymosterol [11]. Cycloartenyl acetate was expoxidized giving 24ξ,25-epoxy-cycloartenyl acetate. This compound was treated with periodic acid in THF giving 25,26,27-tris-nor-cycloartan-24-oxo-3β-yl acetate. This compound was condensed with dimethylamine hydrochloride, and the resulting immonium derivative was immediately reduced *in situ* with $NaBH_3CN$ giving 25-azacycloartanyl (**1**)-acetate, mp 127–130°. Identification of **1**-acetate was achieved by MS and ^1H NMR; the NMR spectrum showed most of the features characteristic for cycloartenyl acetate [13], in addition, the spectrum showed a six-proton resonance at δ 2.323 corresponding to the two methyls bound to the nitrogen atom and a two-proton resonance at 2.343 corresponding to the methylene bound to the nitrogen atom. The MW determined by MS ($M^+ = 471$) corresponded to the proposed structure, in addition specific patterns of fragmentation determined by the N atom were recognized (see details in the Experimental).

Table 1. Sterols of control and 25-azacycloartanol-treated bramble cells

	Control	Treated
Cycloartenol (**2**)	0.5*	36
24-Methylenecycloartanol (**3**)	0.15	0.8
$X_1 + X_2$	tr	7
α- and β-Amyrins	0.5	tr
Cycloeucalenol (**4**)	0.1	0.15
Obtusifoliol (**5**)	0.1	tr
31-Norcycloartenol (**6**)	0	0.1
31-Norlanosterol (**7**)	0	0.1
24-Methylenelophenol (**8**)	0.1	0.2
24-Ethylidenelophenol (**9**)	0.1	0
Isofucosterol (**10**)	12	5.5
Sitosterol (**11**)	70	3
24-Methylenecholesterol (**12**)	2	32
Campesterol (**13**)	14	4
5α-Cholesta-7,24-dien-3β-ol (**14**)	0	0.15
5α-Cholest-7-en-3β-ol (**15**)	0	tr
Desmosterol (**16**)	0	5
Cholesterol (**17**)	tr	4.5
Stanols†	tr	1.6
Total sterols	3.4‡	3.2‡

* As percentage of total sterol.

† 5α-Cholestan-3β-ol; (24ξ)-24-methyl-5α-cholestan-3β-ol; (24ξ)-24-ethyl-5α-cholestan-3β-ol.

‡ mg/g dry wt.

Sterol composition of cells growing on 25-azacycloartanol

Bramble cells were grown in a medium supplemented with **1** (10^{-6} M). Six to 8 weeks were necessary for **1**-treated cells to reach the stationary phase (compared to 4 weeks only for control cells). Higher concentrations of **1** were toxic and inhibited cell growth. The cells were harvested at the stationary phase and the sterols analyzed (Table 1). The sterol compositions of control and **1**-treated cells are shown in Table 2. As shown in Table 1, the total amount of sterols was not significantly altered by treatment of the cells with **1**, but the concentration of 4-desmethyl sterols was much lower in **1**-treated cells (1.75 mg/g dry wt) than in control cells (3.1 mg/g dry wt). Four main results can be pointed out: (i) the amount of C_{10} side-chain sterols (isofucosterol and sitosterol) decreased dramatically in the treated cells (Table 2). Strikingly sitosterol, which is the major sterol in control cells (70%), represented no more than 3% in treated cells (Table 1). (ii) Total C_8 side-chain sterols (cycloartenol, cholesterol, desmosterol, etc.) which were barely detectable in control cells, became very abundant (53%) in treated cells (Table 2). Thus cycloartenol became the major sterol of treated cells; moreover 31-norcycloartenol and 31-norlanosterol which were not present in control cells could be detected in the treated cells (Table 1). (iii) The concentration of C_9 side-chain sterols when expressed as a percentage of total sterols doubled (from 16 to 37%) but when expressed as a percentage of 4-desmethyl sterols increased strongly (from 16 to 66%). For example, 24-methylene cholesterol accumulated and became largely the major 4-desmethylsterol in **1**-treated cells (Table 1). (iv) The relative percentage of $\Delta^{24(25)}$ plus $\Delta^{24(28)}$ sterols (Table 2) increased strongly in **1**-treated cells as shown by the major important increase of the amounts of desmosterol, 24-methylenecholesterol and cycloartenol and the decrease of the amounts of sterols with a saturated side chain (sitosterol, campesterol, etc.) (Table 1).

The results presented above corresponded to the average of three analyses. In one particular experiment, we observed even more drastic changes than those reported here. Thus, in these treated cells the cycloartenol amounted to more than 50% of the total sterols and desmosterol became the major 4-desmethyl sterol (17%)

Table 2. Sterol features occurring in control and 25-azacycloartanol-treated bramble cells

	Control	Treated
Total* C_8 side-chain sterols	0.5†	53†
Total C_9 side-chain sterols	16	37
Total C_{10} side-chain sterols	83	9
Total $\Delta^{24(25)}$- + $\Delta^{24(28)}$-sterols	15	86
4-Desmethyl C_8 side-chain sterols	tr	10 (18)‡
4-Desmethyl C_9 side-chain sterols	16 (16)‡	36 (66)
4-Desmethyl C_{10} side-chain sterols	82 (82)	8.5 (15)
4-Desmethyl $\Delta^{24(25)}$- + $\Delta^{24(28)}$-sterols	14 (14)	42.5 (79)

* 4-Desmethyl-, 4α-methyl- and 4,4-dimethyl-sterols.

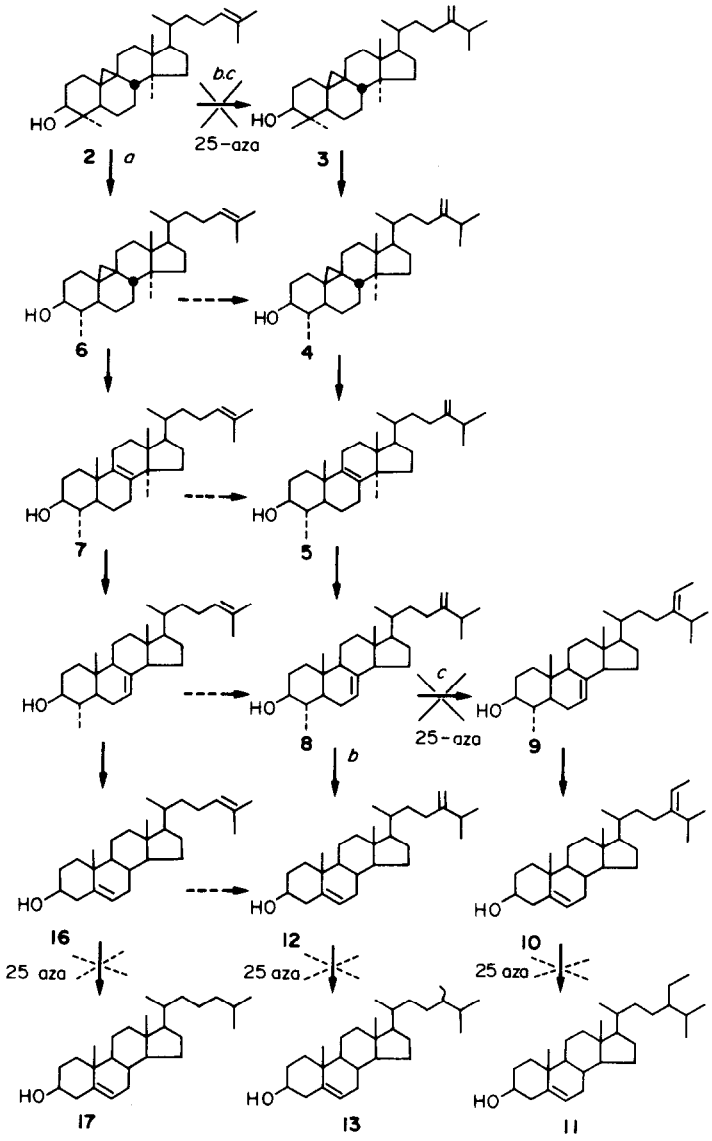
† As % of total sterols.

‡ As % of 4-desmethyl sterols.

whereas sitosterol and isofucosterol were barely detectable. Interestingly the growth of this culture was extremely slow.

DISCUSSION

The results reported in this paper clearly show that bramble cells cultivated in the presence of **1** accumulated sterols with unalkylated lateral chain (cholesterol, desmosterol, cycloartenol, etc.) while the concentration of sterols bearing two additional C atoms at C-24 (C_{10} side-chain sterols: isofucosterol plus sitosterol) was strongly depressed. The fact that upon feeding bramble cells with **1**, cycloartenol and the other C_8 side-chain sterols (cholesterol, desmosterol, 31-norcycloartenol, 31-norlanosterol) accumulated, strongly suggest that **1** inhibited the SAM-cycloartenol-C-24-methyl transferase. The dramatic accumulation of cycloartenol could mean that it is a much poorer substrate for the 31-methyl demethylase than 24-methylenecycloartanol. The important accumulation of 24-methylenecholesterol and the very low amounts of C_{10} side-chain sterols (sitosterol and isofucosterol) suggest that the second SAM-C-28-methyltransferase is also inhibited by **1**. Although 24-methylenelophenol has been shown to be the actual substrate for this enzyme [9], 24-methylenelophenol did not accumulate. This would mean that 24-methylenelophenol is used as efficiently as 24-ethylidenelophenol by enzymes (4α-methyl-demethylase, etc.) transforming them into Δ^5-sterols (mostly 24-methylenecholesterol in the present case). The accumulation of cycloartenol and the absence of accumulation of 24-methylenelophenol would be in agreement with the fact that a pathway leading to 24-methyl sterols normally exists in higher plant cells whereas a route leading to 24-desmethyl sterols is almost non existent. The biosynthetic relationships of the sterols isolated from treated and control cells are shown in Scheme 1. In this scheme three routes leading to



Scheme 1.

C_8 side-chain (route *a*), C_9 side-chain (route *b*), and C_{10} side-chain sterols (route *c*) are represented. In control cells route *a* which is potentially present since traces of cholesterol are detectable, is of minor importance. Of routes *b* and *c* leading respectively to 24-methyl and 24-ethyl sterols, route *c* is the major one. In treated cells, route *a* now becomes an important pathway (presence of 31-norcycloartenol, 31-norlanosterol, accumulation of cholesterol and desmosterol) whereas the biosynthetic flow going through routes *b* and *c* is strongly modified, route *b* being now the favored one.

We have recently shown [12] that SAM-cycloartenol-C-24-methyltransferase and SAM-24-methylene-lophenol-C-28-methyltransferase are strongly inhibited by **1** *in vitro*. This inhibition was quite specific since **1** failed to inhibit significantly other enzymes involved in plant sterol biosynthesis such as 2,3-epoxysqualene-cycloartenol cyclase [14] and cycloeucalenol-obtusifoliol isomerase [15]. Thus the results obtained in the present study confirm that the two methyltransferases are also the target of **1** *in vivo*. The molecular basis of the inhibitors of the two C-methyltransferases has been discussed previously [12]. We have suggested that the protonated form of **1** presents electronic similarities with a putative high-energy intermediate occurring in the methylation reaction. This latter intermediate would possess a carbonium ion at C-25 resulting from the attack of the side chain double bond of cycloartenol on the methyl group of SAM. The theoretical developments concerning properties of molecules that structurally and electronically resemble chemical activated intermediates in catalytis [16] could be invoked to explain the strong inhibitory action of **1** for the C-24 methyltransferases. Another interpretation has been proposed earlier [17] in the case of the inhibition of C-24 methyltransferases by triparanol, a molecule containing a diethylaminoethoxy side chain resembling to the side chain of sterols. According to these authors the lone electron pair of the nitrogen atom, would play the same role as the π electrons of the Δ^{24}-substrate [17, 18] through binding to the sulfonium part of SAM. Such an interpretation could also apply to the inhibition of C-24 methyltransferase by 25-azacycloartanol, although the nitrogen atom of **1** is essentially protonated at the optimal pH (7.4) of the methyltransferases [Benveniste, P., unpublished results].

The second effect of **1** appears to be an inhibition of the Δ^{24}-reductases since $\Delta^{24(25)}$-sterols (cycloartenol, desmosterol) or $\Delta^{24(28)}$-sterols (24-methylenecholesterol) were found to accumulate strongly (Table 2). The mechanism of the reduction of $\Delta^{24(28)}$-sterols is not still fully understood. It is generally postulated that $\Delta^{24(28)}$-sterols isomerize into $\Delta^{24(25)}$-sterols, then the $\Delta^{24(25)}$ bond could be reduced by a reaction involving a *trans*-antiperiplanar addition of H^+ (from the medium) and H^- from NADPH [1]. The resemblance of this reaction with the C-methylation has been emphasized [17]. Both reactions involve first the addition of an electrophilic component (H^+ or Me^+) leading to the formation of a carbonium ion at C-25. This latter would then react with an H^- ion provided by NADPH in the case of the Δ^{24} reductase or with a suitable nucleophilic group of the enzyme in the case of the C-24 methyltransferase [1]. Thus the protonated form of **1** would present similarities not only with the C-25 carbonium ion involved during the C-24 methylation step but also with the C-25 carbonium ion involved during the postulated $\Delta^{24(25)}$ reduction step and these considerations explain why **1** behaves also as a good inhibitor of the Δ^{24}-reduction of Δ^{24}-sterols.

Finally the results presented here show clearly that it is possible to modify the extent of alkylation of the sterols in bramble cells grown *in vitro*. Since these cells can be grown permanently in the presence of **1**, it will be possible to study the influence of the disappearance of C_{10} side-chain sterols and the accumulation of C_8 side-chain sterols on the structure and function of their plasma membranes.

EXPERIMENTAL

Most of the techniques used in the present work have been described in detail previously [19]. The RR_fs (OV-17, cholesterol, RR_f 1.0) on GLC for the acetates of the 4-desmethyl sterols isolated in this study were: sitosteryl (**11**)-acetate, 2.05; isofucosteryl (**10**)-acetate, 2.24; campesteryl (**13**)-acetate, 1.67; 24-methylenecholesteryl (**12**)-acetate, 1.73; cholesteryl (**17**)-acetate, 1.29; desmosteryl (**16**)-acetate, 1.57; 5α-cholesta-7,24-dien-3β-yl (**14**)-acetate, 1.80; 5α-cholest-7-en-3β-yl (**15**)-acetate, 1.52. The RR_fs (SE-30, cholesterol, RR_f 1.0) on GLC for the acetates of the 4α-methyl sterols and of the 4,4-dimethylsterols isolated in this study were: 24-methylenelophenyl (**8**)-acetate, 2.08; 31-norlanosteryl (**7**)-acetate, 1.66; 31-norcycloartenyl (**6**)-acetate, 1.87; cycloeucalenyl (**4**)-acetate, 2.15; obtusifoliyl (**5**)-acetate, 1.88; 24-methylenecycloartanyl (**3**)-acetate, 2.52; cycloartenyl (**2**)-acetate, 2.21; X_1-acetate, 2.02; X_2-acetate, 2.20. The RR_fs (OV-17, cholesterol, RR_f 1.0) on GLC for the acetates of pentacyclic triterpenes present in bramble cells were: α-amyrin acetate, 2.37; β-amyrin acetate, 2.10.

Plant material. Suspension cultures of bramble cells were grown under continuous white light at 25° on a synthetic sterile medium as described previously [9]. 25-Azacycloartanol (0.5 mg/l.) was added in soln in EtOH to the culture medium. The drug was sterilized before use by filtration through a Millipore (0.45 μm) filter.

Analytical procedure. The isolation of 4,4-dimethyl-, 4α-methyl- and 4-desmethylsteryl acetates has been described previously [9]. Each of three classes of acetates was analysed by GLC, and the total amount of sterols present in each class was quantified. Analytical argentation TLC, in which cyclohexane–toluene (3:2) was the developing solvent and migration was for 15 hr, was performed on each class of steryl acetate and the bands obtained were analysed by GLC. There were 3 bands of 4,4-dimethylsteryl acetates in the case of both control bramble cells and treated cells, corresponding in order of decreasing polarity to 24-methylenecycloartanyl (**3**)-acetate, cycloartenyl (**2**)-acetate and a mixture of α- and β-amyrin acetates. In the case of treated cells, there was one additional band at a R_f intermediate between R_fs of **2** and **3**-acetates. This band contained the acetates of two tetracyclic triterpenes X_1 and X_2 [20]. There were 3 bands of 4α-methyl steryl acetates from control bramble cells, corresponding in order of decreasing polarity to 24-methylenelophenyl (**8**)-acetate, a mixture of cycloeucalenyl (**4**)-acetate and obtusifoliyl (**5**)-acetate, and 24-ethylidenelophenyl (**9**)-acetate; and there were 3 bands also for **1**-treated cells, the first 2 bands at the same R_fs as in the control cells, the third at an R_f slightly higher than the R_f of the less polar band from the control cells. The first two bands contained respectively **8**-acetate and a mixture of **4**- and **5**-acetate, the third band did not contain **9**-acetate but a mixture of 31-norcycloartenyl (**6**)-acetate and of 31-norlanosteryl (**7**)-acetate. There were 3 bands of 4-desmethyl steryl acetates from control bramble cells, corresponding in order of decreasing polarity to 24-methylenecholesteryl (**12**)-acetate, isofucosteryl (**10**)-acetate, and a mixture of campesteryl (**13**)- and sitosteryl (**11**)-acetates. From

1-treated cells there were also 3 bands. The first band was by far the major one and contained mostly **12**-acetate; the second, a mixture of **10**-acetate, of desmosteryl (**16**)-acetate and of 5α-cholesta-7,-24-dien-3β-yl (**14**)-acetate; the third, a mixture of cholesteryl (**17**)-acetate, of 5α-cholest-7-en-3β-yl (**15**)-acetate, of campesteryl (**13**)-acetate and of sitosteryl (**11**)-acetates. **14**-acetate could be separated from **10**-acetate and **16**-acetate by argentation TLC using commercial unwashed $CHCl_3$ as the developing solvent. **15**-acetate could be separated from **13**-, **11**-, and **17**-acetates by argentation TLC using free EtOH–$CHCl_3$ as the developing solvent. All the sterols isolated from **1**-treated cells were identified by comparing their RR_f on GLC, their MS and ^1H NMR spectra with those of authentic samples (in the case of **2**-, **3**-, **4**-, **5**-, **6**-, **7**-, **8**-, **10**-, **11**-, **12**-, **13**-acetates) or with those reported in ref. [21] (**15**-, **16**-, **17**-acetates) and in ref. [22] (**14**-acetate).

Chemical synthesis of 25-azacycloartanol (**1**). 24ξ,25-*Epoxycycloartenyl* (**18**)-*acetate*. Cycloartenyl (**2**)-acetate (500 mg) was treated by *p*-nitroperbenzoic acid in the usual way [23]. After crystallization **18**-acetate (435 mg) was obtained mp 141–143°, lit. [24] 143.5–144°.

25,26,27-*Tris-nor-cycloartan*-24-*oxo*-3β-*yl* (**19**)-*acetate*. (**18**)-acetate (340 mg) dissolved in dry Et_2O (100 ml) was treated with dry H_5IO_6 (220 mg) in soln in THF (1.5 ml) for 5 hr at room temp. The reaction was stopped by addition of a 10% soln of $Na_2S_2O_3$ in H_2O. After extraction the reaction products were submitted to TLC using CH_2Cl_2 as developing solvent, and a mixture of two products (2/3, 1/3), respectively **19**-acetate and 24-oxo-cycloartan-3β-yl (**20**)-acetate, was recovered. **19**-Acetate was separated from **20**-acetate by continuous TLC using CH_2Cl_2 as developing solvent. **19**-acetate: 97% pure (GLC, SE-30), mp 167–170°, lit. [25] 155–157°; MS *m/e* (rel. int.): 442 (M^+) (3), 427 (7), 382 (78), 367 (100), 339 (43), 297 (52), 260 (40). **20**-Acetate: MS: 484 (M^+) (5), 469 (3), 424 (100), 409 (93), 302 (24), 297 (75).

N,N-*Dimethyl*-24-*amino*-25,26,27-*tris-nor-cycloartan*-3β-*yl* (**1**)-*acetate*. The mixture of **19**- and **20**-acetates dissolved in MeOH, THF (2:1) was treated with $(Me)_2NH$, HCl (180 mg), pH was adjusted to about 7, then $NaBH_3CN$ (24 mg) was added. The reaction was performed for 24 hr at room temp. and under stirring. After extraction with Et_2O, the products of the reaction were submitted to TLC using $CHCl_3$–MeOH–NH_4OH (175:25:0.6) as developing solvent. Four bands were observed, the band corresponding to the less polar compound had the same R_f as the starting product and corresponded to unreacted **20**-acetate, this band was not present when pure **19**-acetate was used as substrate of the reaction. The major band (R_f 0.28) was shown to contain **1**-acetate (65 mg). 1-Acetate: 98% pure (GLC, SE-30 1%), mp 127–130 (from Me_2CO). MS *m/e* (rel. int.): 471 (M^+) (100), 456 (35), 428 (6), 411 (31), 396 (7), 366 (14). ^1H NMR (in $CDCl_3$): δ 0.342 (1H, *d*, $J = 4$ Hz, C-19), 0.571 (1H, *d*, $J = 4$ Hz, C-19), 0.846 (3H, *s*, C-30), 0.878 (3H, *d*, $J = 4.5$ Hz, C-21), 0.887 (6H, *s*, C-18, C-32), 0.957 (3H, *s*, C-31), 2.056 (3H, *s*, OAc), 2.323 (6H, *s*, C-26, -27), 2.343 (2H, *t*, $J = 8$ Hz, C-24), 4.563 (1H, *dd*, C-3αH).

Authentic materials. Cycloartenol (**2**) was the kind gift of Dr. A. S. Narula (Canberra, Australia). Cycloeucalenol (**4**) was extracted from tallow wood (*Eucalyptus microcorys*). Obtusifoliol (**5**) was kindly supplied by Professor Gonzales-Gonzales (Tenerife, Las Canarias, Spain). 31-Norlanosterol (**7**) was a gift from Professor Goutarel (Gif sur Yvette, France). 31-Norcycloartenol (**6**) was supplied by Dr. L. N. Standifer (Tucson, U.S.A.). Desmosterol (**16**) was supplied by Dr. Lu Bang (Strasbourg, France). 24-Methylenecycloartanol (**3**) was a gift from Dr. Itoh (Tokyo, Japan).

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