(0.316 mole) of phosgene, when allowed to react with 100 g. (1.11 moles) of 1,3-dihydroxy-2-propanone, afforded 61.2 g. of a mixture of IV and V. The crude product was extracted with boiling ether (6 × 200 ml.) to remove the bisester. The ether-insoluble material was recrystallized three times from 95% EtOH, yielding 25.0 g. (32.3%) of analytically pure IV: m.p. 121–126°; $\nu_{max.}^{mineral\ oil}$ 3356 (OH) and 1739 cm.$^{-1}$ (carbonyl); $\lambda_{max.}^{EtOH}$ 219, 266, 273, and 280 nm. (ϵ 7570, 1220, 1630, and 1390).

Anal.—Calc. for $C_{12}H_{14}O_6$: C, 56.69; H, 5.55. Found: C, 56.64; H, 5.69.

The combined ethereal extracts were evaporated *in vacuo*. The solid residue was recrystallized five times from 95% EtOH, affording pure V: m.p. 101–102°; $\nu_{max.}^{CHCl_3}$ 1751 cm.$^{-1}$ (carbonyl); $\lambda_{max.}^{EtOH}$ 218, 264, 271, and 278 nm. (ϵ 1420, 2320, 3100, and 2590); mass spectrum, *m/e* (relative intensity) 418 (6.3), 325 (5.6), 237 (50), 143 (41), 121 (92), and 94 (100); isotope distribution calc. for $C_{21}H_{22}O_9$, *m/e* (relative intensity) 418 (100), 419 (23.3), 420 (4.38), found 418 (100), 419 (23.5), 420 (4.30).

Anal.—Calc. for $C_{21}H_{22}O_9$: C, 60.28; H, 5.30. Found: C, 60.15; H, 5.05.

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Structure Elucidation of Maytenonic Acid, a New Triterpene from *Maytenus senegalensis* (Celastraceae)

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Abstract □ Evidence for the structure of a new triterpene from *Maytenus senegalensis*, named maytenonic acid, is presented. The structure was postulated mainly on the basis of interpretation of IR, NMR, and mass spectral data.

Keyphrases □ Maytenonic acid, triterpene from *Maytenus senegalensis*—structure elucidation □ *Maytenus senegalensis* (Celastraceae)—structure elucidation of maytenonic acid □ Spectroscopy, IR, NMR, mass—structure elucidation of maytenonic acid from *Maytenus senegalensis*

Previously, the isolation and identification of β-amyrin, lupenone, β-sitosterol, β-sitosterol xyloside, wilforine, dulcitol, and two new triterpenes from *Maytenus senegalensis* (Lam.) Excell. were reported (1). Extracts and fractions from the plant were shown to have reproducible activity against the 9KB carcinoma of the nasopharynx in cell culture, as well as the L-1210 leukemia and PS leukemia tumor systems in mice. Dulcitol was shown to be responsible for a part of the antitumor activity. At this time, evidence is presented for the structure elucidation of one of the triterpenes previously isolated, triterpene A (1).

DISCUSSION

Maytenonic acid (I) (triterpene A), m.p. 262°, $[\alpha]_D^{26}$ −32 (c, 0.5, MeOH) was shown to have a molecular formula of $C_{30}H_{48}O_3$ by

Table I—Mass Spectrometric Data for Maytenonic Acid[a]

m/e	Percent	*m/e*	Percent
456	8.7	163	32.6
441	6.5	155	63.5
410	4.3	135	22.3
395	2.0	121	33.4
371	4.3	109	100.0
273	32.6	95	61.8
250	16.2	81	52.7
221	8.7	69	42.8
218	13.0	55	60.5
205	6.5	43	52.7
189	10.0	41	34.5

[a] Spectra were determined using a mass spectrometer LKB-9000 (LKB Produkter, Stockholm, Sweden) at 70 e.v.

Table II—High-Resolution Mass Spectral Measurements of Selected Ions of Maytenonic Acid[a]

Formula	Calculated *m/e*	Observed *m/e*
$C_{30}H_{48}O_3$	456.3603	456.3628
$C_{29}H_{46}O$	410.3548	410.3573
$C_{28}H_{43}O$	395.3314	395.3315
$C_{25}H_{39}O_2$	371.2950	371.2968
$C_{19}H_{29}O$	273.2218	273.2229
$C_{16}H_{26}O_2$	250.1933	250.1952
$C_{15}H_{25}O$	221.1905	221.1943
$C_{14}H_{21}O_2$	221.1541	221.1580
$C_9H_{15}O_2$	155.1072	155.1074
C_9H_{13}	121.1017	121.1023
C_8H_{13}	109.1017	109.1003

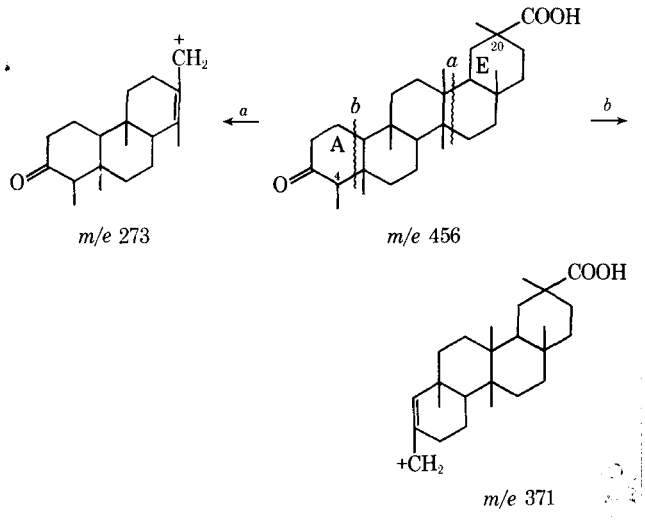
[a] Spectra were obtained on a double-focusing mass spectrometer MS 9 (Allied Electrical Industries Ltd., Manchester, England).

high-resolution mass spectrometric measurements (Tables I and II). These data, together with a positive Liebermann–Burchard color test, showed the compound to be a triterpene. The IR spectrum of I in mineral oil exhibited two separate bands of nonconjugated carbonyl groups at 5.7 and 5.83 μ, together with a broad absorption band between 2.8 and 3.8 μ, corresponding to hydrogen bonding of an OH group. The IR spectrum of I in chloroform was similar to that in mineral oil but the broad band was shifted toward higher wavelengths (3.0–4.0 μ) and both carbonyl bands appeared at about 5.81 and 5.85 μ.

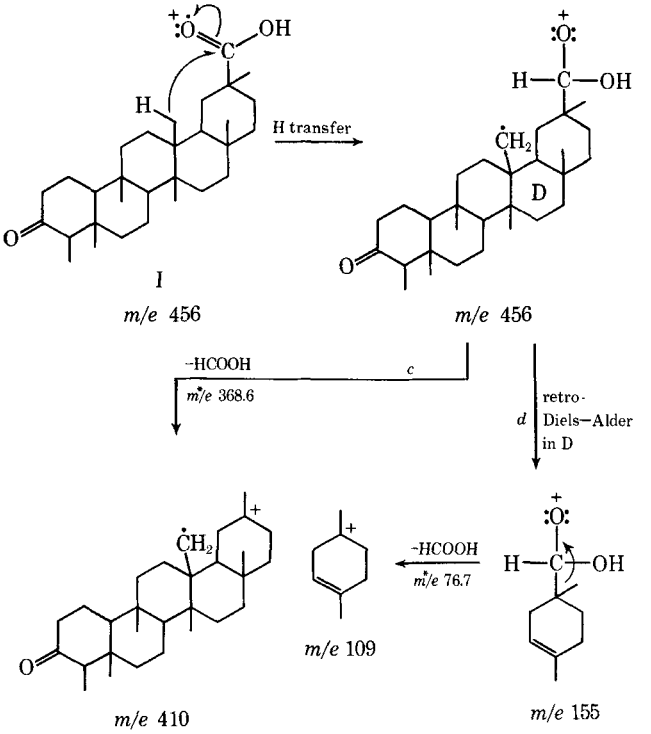
These spectral characteristics indicated the presence of either two keto and one hydroxyl group, or a keto and a carboxy group. To distinguish between these two possibilities, an attempt was made to extract I from chloroform solution with a 5% solution of sodium hydroxide; however, the compound did not appear in the aqueous fraction after acidification as would be expected of an acid. That, in fact, a carboxy group was present in the molecule was later discovered by gently warming I in dimethyl sulfoxide with aqueous sodium hydroxide followed by cooling. The material that precipitated from solution was the water-insoluble sodium salt of an acid, m.p. >300°, as shown by its IR spectrum (mineral oil) in which the original carbonyl band at 5.83 μ was shifted to 6.2 μ.

An examination of the mass spectrum of maytenonic acid (Table I) afforded evidence that I belonged to the friedelane series of triterpenes. Earlier, it was shown (2, 3) that the peaks at *m/e* 273 and 341 for 3-keto friedelane arise by retro-Diels–Alder reaction, together with a loss of one hydrogen, and they are very characteristic for this series of triterpenes. The presence of peaks at *m/e* 273 and 371 (Table II) in the mass spectrum of I provided not only the evidence concerning the carbon skeleton of I but also indicated that one oxygen function, *i.e.*, the carbonyl group, was in the A ring and that the acid function was in the terminal E ring (Scheme I).

The NMR spectrum of I confirmed the mass spectral evidence for the friedelane type of triterpene since it lacked the signals for



Scheme I



Scheme II

olefinic protons and it exhibited a methyl doublet at 0.85 δ due to the methyl group at C_4. Moreover, integration showed the presence of seven methyl groups, again confirming that I was a keto carboxylic acid, since there would be eight methyl groups present if I were a diketo hydroxy compound or it would show a CH_2OH absorption if the hydroxyl group was located at one of the methyl groups. Finally, the NMR spectrum also gave evidence concerning the position of the acid group on ring E. Tursch *et al.* (4, 5) studied the NMR spectra of a large series of triterpenes and found that the absorption of a C-methyl group geminal to a carboxyl group occurs downfield from all other C-methyl groups at about 1.25 δ. The NMR spectrum of I contained one methyl absorption at 1.25 δ, thus placing the carboxylic function with all due probability at C_{20}.

With this structure in hand, it was possible to interpret further the strong peaks in the mass spectrum of maytenonic acid (Tables I and II) as shown in Scheme II.

If the interpretation of the transfer of one hydrogen from the methyl group at C_{13} (pathways *c* and *d*) is correct, then the carboxyl group at C_{20} should be *cis* oriented with respect to the methyl group at C_{13}.

Further studies are underway to confirm the structure of maytenonic acid as I, as well as to establish the stereochemistry of the molecule.

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This paper is dedicated to Professor Dr. h. c. mult. Kurt Mothes, Emeritus Director, Institute for the Biochemistry of Plants, German Academy of Science of Berlin, on his 70th birthday.

Collaborative Study of Aerobic Media for Sterility Testing by Membrane Filtration

FRANCES W. BOWMAN, MACK WHITE, and MIRIAM P. CALHOUN

Abstract □ The USP XVIII and NF XIII (first supplement) have replaced the sterility test medium, fluid Sabouraud, with a soybean-casein digest medium. A collaborative study performed by 12 laboratories showed that soybean-casein digest medium is superior to fluid Sabouraud medium for sterility testing by membrane filtration.

Keyphrases □ Aerobic media for membrane filtration sterility testing—collaborative study □ Soybean-casein digest medium—collaborative study of use in membrane filtration sterility tests □ Membrane filtration sterility testing—collaborative study on soybean-casein digest medium

The Food and Drug Administration has been using Sabouraud liquid medium USP (pH 5.7) for testing the sterility of antibiotics since 1964 (1). Prior to that time, a modified Sabouraud medium (also pH 5.7) was used (2). The primary reason for using this medium in the sterility test was to detect the presence of fungi rather than bacteria. A few bacteria found as contaminants of antibiotics did not grow at pH 5.7 but were recovered in thioglycollate medium (pH 7.1). Since the sterility test is intended to detect as many microorganisms as possible, it is patently undesirable to use a medium, such as fluid Sabouraud at pH 5.7, that inhibits certain bacteria. To eliminate the use of a selective medium for the sterility test, the USP XVIII and the first supplement to NF XIII replaced fluid Sabouraud USP XVII with a soybean-casein digest (SBCD) medium which has a pH of 7.3 ± 0.2. However, before implementing any change in the sterility tests for antibiotics as required in the *Antibiotic Regulations*, a collaborative study was performed to compare the growth-promoting qualities of SBCD medium to those of fluid Sabouraud. Twelve laboratories participated in this study[1].

Since preliminary work indicated that fluid Sabouraud medium at pH 7.0 supports the growth of fungi without inhibiting bacteria, this medium was also included in the study. All three media were manufactured by each of two companies (designated as Manufacturers A and B) for use in this study. A protocol containing detailed instructions and the media to be used in the study were supplied to each collaborator (3).

[1] Biological Safety Control, Becton, Dickinson and Co., Raleigh, N. C.; Bristol Laboratories, Syracuse, N. Y.; Difco Laboratories, Detroit, Mich.; Food and Drug Administration, National Center for Antibiotic Analysis, Sterility Testing Branch; Food and Drug Directorate Laboratories, Ottawa, Canada; Lederle Laboratories, Pearl River, N. Y.; Eli Lilly and Co., Indianapolis, Ind.; Laboratory of Control Activity, Division of Biological Standards, National Institutes of Health, Bethesda, Md.; Parke, Davis & Co., Detroit, Mich.; Chas. Pfizer & Co., Inc., Brooklyn, N. Y.; E. R. Squibb & Sons, Inc., New Brunswick, N. J.; and Wyeth Laboratories Inc., West Chester, Pa.

EXPERIMENTAL

The procedure used was essentially the same as that used for testing the sterility of antibiotics (4). The design of the study was similar to that used in *Reference 5*.

Organisms—Cultures or spore suspensions of the nine microorganisms used in the study were supplied by the authors. Dilutions of inocula were selected to simulate low levels of contamination that might possibly be encountered in a contaminated pharmaceutical preparation being tested for sterility. The following microorganisms were employed: *Bacillus subtilis* ATCC 6633 (spores), *Staphylococcus aureus* ATCC 6538 P; *Aspergillus niger* ATCC 6275 (spores), *Bacillus circulans* PCI 260 (spores), *Bacillus* sp. PCI 208 (spores), *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 10536, and *Bacillus* sp. PCI 258 (spores).

Media—Instructions for reconstitution and sterilization of the media were as follows: Weigh 120 g. of the dehydrated medium and rehydrate by adding 4 l. of distilled water. If necessary, warm the mixture to complete solution. When the medium is in solution, dispense it in 100-ml. portions to each of 36 (38 × 200-mm.) screw-capped tubes. Label each tube as to contents. Sterilize the tubes of media in an autoclave at 121° for 20 min. The autoclave temperature should be reached within 10 min.

Each collaborator prepared a total of 216 labeled tubes, comprising 36 tubes each of six media (SBCD medium A and B, Sabouraud medium pH 5.7 A and B, and Sabouraud medium pH 7.0 A and B).

Incubation Time—Incubation time was limited to 2, 5, and 7 days since the collaborative study of insulin (4), in which samples were incubated 7, 10, and 14 days, had shown that 7 was the maximum number of days required for the recovery of microorganisms where the membrane filtration procedure was used.

The following protocol was supplied to and followed by the collaborators: For each of the nine microorganisms, prepare about 150 ml. of a stock organism suspension containing approximately 100 colony-producing units (CPU) per 20 ml. of sterile 0.1% peptone solution and dispense 20 ml. into seven sterile bottles. From the remaining stock, prepare a low-level inoculum of approximately 5 CPU/20 ml. by adding 1 ml. to 19 ml. of sterile 0.1% peptone solution in each of seven bottles. Immediately perform the membrane filtration test on the contents of each of the 14 bottles as follows: Filter the entire contents of one bottle through a 0.45-μ membrane filter. Rinse the filter by filtering 100 ml. of sterile 0.1% peptone solution through it. Cut a circular disk approximately 17.5