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Microwave-assisted preparation of cyclic ketals from a cineole ketone as potential cosmetic ingredients: solvent-free synthesis, odour evaluation, in vitro cytotoxicity and antimicrobial assays

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Abstract

Some cyclic ketals derived from (+)1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-one were obtained in excellent yields by microwave activation under solvent-free conditions, as a 'green chemistry' procedure. The results obtained using acidic alumina containing 7% *p*-toluenesulfonic acid, as mineral support, are reported and compared with those obtained by classical methods. The new compounds were tested for their olfactive character and for a potential cosmetic use. In vitro skin cytotoxicity tests were carried out on the most promising compounds, by using NCTC 2544 human keratinocytes as target cells. They all displayed slight cytotoxic effects which were one order of magnitude lower than those found with sodium dodecylsulphate positive control. Two compounds that resulted interesting as toothpaste aromas, were submitted to antimicrobial assays and showed their activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus hominis*, *Propionibacterium acnes* and *Candida albicans*.

Keywords: Cyclic ketals; Microwaves and solvent-free conditions; Green chemistry; Cosmetic odourants; In vitro cytotoxicity tests; Antimicrobial activity

1. Introduction

Microwave irradiation is becoming popular as a rapid and clean synthetic method especially when coupled to solvent-free techniques (Bram et al., 1992; Loupy et al., 1998; Varma, 1999).

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These methodologies lead to increased safety, cost reduction and respect of the environment together with their essential interest in synthesis due to noticeable efficiency (improvement in yields, obtaining purer products within shorter reaction times).

The cyclic ketals are classically prepared by reaction of a carbonyl group with diols in the presence of an acidic catalyst, in benzene or toluene as solvents and subsequent azeotropic removal of water. Recently, several publications described the use of microwaves in acidic media, as simplified and cleaner procedures, to perform a lot of acetalizations. They concern either montmorillonites as an acidic solid support (Csiba et al., 1993; Li et al., 1997; Kad et al., 1998) or *p*-toluenesulfonic acid as a catalyst (Matloubi Moghaddam and Sharifi, 1995; Pério et al., 1997; Wang et al., 1997; Kalita et al., 1998; Pourjavadi and Mirjalili, 1999).

In pursuing our studies on cineole derivatives which are interesting as odourants (Mariani et al., 1995a,b) and taking into account that some terpenyl ketals have a pleasant odour (Buchbauer and Lux, 1991), we have synthesized and tested for their olfactive character (Anaç and Talinli, 1993) some new 1,3 dioxolanes (**2a**–**e**) and 1,3 dioxanes (**3a**–**c**) starting from the cineole ketone (+)1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-one **1**. In the present study a simple, efficient, fast and environmentally friendly method for the synthesis of these compounds, using microwave irradiation under solvent-free conditions, is reported (Bram et al., 1992; Loupy et al., 1998; Varma, 1999; Csiba et al., 1993). In order to evaluate their potential cosmetic use, all the compounds were submitted to odour evaluation and those resulting more promising (**2a**, **2c**, **3a** and **3b**) were then selected to assess their skin cytotoxicity and antimicrobial properties.

Three compounds, **2a**, **2c** and **3b**, were evaluated for their potential skin damaging activity whereas the compound **3a**, being poorly soluble in culture media, was not tested.

Two in vitro tests, which are widely employed as cost-effective and rapid methods in the prescreening phase of the development of new drugs and other chemicals, were used.

The Neutral Red Uptake (NRU) test is based on the ability of viable cells to incorporate and bind neutral red, a vitally weak cationic dye. It penetrates the cell membranes by non-ionic passive diffusion and concentrates in the lysosomes, where it binds by electrostatic hydrophobic bonds to various groups of the lysosomal matrix. The MTT assay is based on the reduction of the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to a purple coloured MTT-formazan precipitate by mitochondrial dehydrogenase enzymes, which are still active only in the surviving cells. Both assays were established in a cell culture model represented by the NCTC 2544 human keratinocyte cell line which, being well characterized with regards to drug metabolism (Cotovio et al., 1997), can be considered as a suitable system to in vitro skin toxicology studies.

Two compounds, **2c** and **3b**, resulted interesting as aromas for oral care products, were tested for their antimicrobial activity, in order to evaluate a possible application in toothpastes.

2. Materials and methods

2.1. Apparatus

Microwave irradiation was carried out using a Synthewave™ S402 from Prolabo (Fontenay-Sous-Bois, France). IR spectra were registered with a Perkin–Elmer 398 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian-Gemini 200 instrument (200 MHz) with TMS as an internal standard; chemical shifts are reported as δ (ppm) relative to TMS. Elemental analyses for C and H were performed on CE Instruments-EA 1110 CHNS-O. Melting points were determined on a Büchi 510 apparatus and were uncorrected.

2.2. Chemicals

Acidic alumina, Na-thiosulphate, toluene and diethylether were supplied by Merck (Darmstadt, Germany); montmorillonite, ethylene glycol, 1,2-propanediol, 1,3-propanediol, (±)2,3-butanediol, (±)1,2-butanediol, (±)1,3-butanediol, 2,2-

dimethyl-1,3-propanediol, (±)2,4-pentanediol, *p*-toluenesulfonic acid, tetraglycol, Dulbecco's modified Eagle's medium (DMEM), MTT and sodium dodecylsulphate (SDS) were supplied by Sigma–Aldrich (Milan, Italy); L-glutamine and Neutral Red were from ICN Biomedicals Inc., (Costa Mesa, CA, USA); trypsine–EDTA was from Gibco BRL (Paisley, Scotland); foetal calf serum was from Mascia Brunelli (Milan, Italy); cell culture flasks and 96-wells plates were from Costar (Cambridge, UK). TSA and TSB (Tryptone soya agar and Tryptone soya broth) and SPS Agar (Sulphite polimixin sulphadiazine agar) were provided by Oxoid/Unipath (Milan, Italy); fluid thioglycollate medium and Tryptone by Difco (Surrey, UK); lecithin from eggs, Polysorbate 80, L-histidine, Tween 80 by BBL (Franklin Lakes, USA).

2.3. Cell line

Normal human keratinocyte cell line NCTC 2544 was provided by Interlab Cell Line Collection, (Genoa, Italy).

2.4. Test microorganisms

Pseudomonas aeruginosa (ATCC 9027, 6.67×108 CFU/ml), *Escherichia coli* (ATCC 8739, 6.08×109 CFU/ml), Clinical isolate (n. H1-6336152.140300) *Staphylococcus hominis* (ST/H1-6336152, 1.53×108 CFU/ml), *Candida albicans* (ATCC 10231, 2.46×107 CFU/ml), *Propionibacterium acnes* (ATCC 11827, 9.75×107 CFU/ml) were supplied by Biogenetics Srl (Padova, Italy).

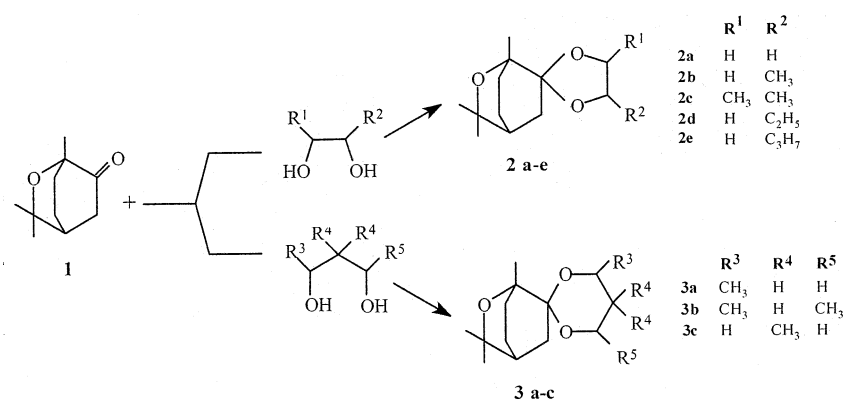
2.5. Chemistry

The equipment used for microwave irradiation was a monomode reactor (Synthewave 402-Prolabo) which, in addition to the interest of wave focussing which allows for a homogeneous electric field, presents a number of advantages: temperature measurement by infrared detection, temperature control by power modulation between 15 and 300 W, computer monitoring of the reaction and mechanical stirring with adjustable rotation speed (Loupy et al., 1998).

The cineole ketals, **2a**–**e** and **3a**–**c**, were prepared under dry conditions and microwave activation by acid-catalyzed condensation of compound **1** with several aliphatic diols (Scheme 1).

A number of experiments were performed in order to evaluate the most efficient catalytic conditions (Table 1) including the use of neat *p*-toluenesulfonic acid (PTSA) in the absence or presence of toluene or when impregnated on acidic alumina in 'dry media'; as an example, the data related to the compound **2b** are reported. The best results were obtained using PTSA in toluene or impregnated on acidic alumina (Loupy et al., 1992).

In Table 2 and Table 3 the main results for all the derivatives are given and compared to those obtained by the classical method (*p*-toluenesulfonic acid, toluene and Dean Stark apparatus).



Scheme 1.

Table 1
Syntheses of compound **2b** at 110 °C[a] under several conditions by reacting **1** and two equivalents of 1,2-propanediol

Method		Time (min)	Yield[b] %
Montmorillonite	MW	30	0[c]
Acidic alumina	MW	30	0[d]
PTSA	MW	30	65
Acidic alumina/PTSA	MW	30	78
Acidic alumina/PTSA	Δ	30	25[e]
Acidic alumina/PTSA	Δ	300	27[f]
Toluene/PTSA	MW	15	90
Toluene/PTSA	Δ	360	30[f]

MW, Microwave activation; Δ, Classical heating.
[a] Final temperature evaluated by IR detection for microwave methods, by digital thermometer for classical methods.
[b] Yield refers to pure isolated product.
[c] Degradation compounds.
[d] Starting materials.
[e] Complement = starting materials.
[f] Complement = starting materials + degradation compounds.

In order to check the possible intervention of not purely thermal specific microwave effects, the syntheses of compounds **2b**, **2c** and **3b** were carried out in dry media, under the same conditions as under microwave irradiation (vessel, time, temperature), but in a thermostated oil bath and the results then compared. An accurate control in the rise in temperature inside the reaction medium was carried out for both conditions (Tables 1–3).

As an example, the temperature rise profiles obtained for compound **2b** are reported in Fig. 1.

2.5.1. General procedure for the microwave mediated syntheses under dry conditions of compounds *2a–e* and *3a–c*

The acidic support (6 g of acidic alumina impregnated with 7% *p*-toluenesulfonic acid; ketone/support 1/4) was added to a solution of cineole ketone **1** (10 mmol) and the appropriate diol (20 mmol) in diethylether (10 ml). The mixture was stirred and the solvent was evaporated under reduced pressure. The resulting solid was placed into a Pyrex tube, introduced into the microwave reactor and irradiated for 30 min at 110 °C, maintaining this temperature by power modulation with a maximum power of 300 W. After cooling to room temperature, 10 ml of diethylether were added, the mixture was filtered with Gooch filter and the solvent evaporated. The crude product was purified by silica gel column chromatography (cyclohexane/diethylether 7/3 v/v) and by bulb-to-bulb distillation in vacuo or by recrystallization from a suitable solvent.

2.5.2. General procedure for the classical syntheses of compounds *2a–e* and *3a–c*

A solution of cineole ketone **1** (10 mmol) and the appropriate diol (20 mmol) in anhydrous toluene (50 ml) containing *p*-toluenesulfonic acid (10% by weight of ketone) was refluxed in a Dean Stark apparatus for 6 h. After cooling to room temperature, the reaction mixture was washed with a solution of sodium bicarbonate, then dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue obtained was purified as above.

The IR and ^{1}H-NMR spectral data of all the synthesized compounds were in agreement with the proposed structures as isomeric mixtures. Microanalysis for C and H were within $\pm 0.3\%$ of the theoretical values.

2.6. Odour evaluation

All the synthesized compounds were submitted to an odour evaluation test. The samples, diluted in dipropylene glycol 5% w/v, were evaluated on paper after solvent evaporation: either immediately, after 10 min, 6 and 24 h. The note, the reference note, the intensity, the evolution, the persistence and the potential cosmetic uses were investigated. The olfactive properties of the most interesting odourants (compounds **2a**, **2c**, **3a**, **3b**) are reported in Table 4.

2.7. Skin cytotoxicity evaluation

Normal human keratinocyte cell line NCTC was grown in DMEM medium supplemented with 10% foetal calf serum and 2% L-glutamine, at 37 °C, in a humidified atmosphere of 5% CO_2/95% air. The medium was changed every 2–3 days and, when the original flask was approximately 80% confluent, the cells were subcultured by trypsin-EDTA digestion.

Table 2
Syntheses of compounds **2a–e** at 110 °C[a] in the presence of PTSA

Compound	Method		Time (min)	Yield[b]%	bp °C (mmHg)
2a	Acidic alumina	MW	30	72	55–60 (0.8)
	Toluene	MW	15	80	
	Toluene	Δ	360	4[c]	
2b	Acidic alumina	MW	30	78	60–65 (0.7)
	Toluene	MW	15	90	
	Toluene	Δ	360	30[c]	
	Acidic alumina	Δ	30	25[d]	
	Acidic alumina	Δ	300	27[c]	
2c	Acidic alumina	MW	30	75	64–68 (0.8)
	Toluene	MW	15	90	
	Toluene	Δ	360	54[c]	
	Acidic alumina	Δ	30	15[d]	
2d	Acidic alumina	MW	30	82	65–69 (0.6)
	Toluene	MW	15	85	
	Toluene	Δ	360	30[c]	
2e	Acidic alumina	MW	30	78	73–75 (0.5)
	Toluene	MW	15	83	
	Toluene	Δ	360	43[c]	

MW, Microwave activation; Δ, Classical heating.
[a] Final temperature evaluated by IR detection for microwave methods, by digital thermometer for classical methods.
[b] Yield refers to pure isolated product.
[c] Complement = starting materials + degradation compounds.
[d] Complement = starting materials.

Table 3
Syntheses of compounds **3a–c** at 110 °C[a] in the presence of PTSA

Compound	Method		Time (min)	Yield[b]%	bp °C (mmHg) mp °C
3a	Acidic alumina	MW	30	77	65–68[e]
	Toluene	MW	15	86	
	Toluene	Δ	360	18[c]	
3b	Acidic alumina	MW	30	60	71–74 (0.8)
	Toluene	MW	15	70	
	Toluene	Δ	360	12[c]	
	Acidic alumina	Δ	30	19[d]	
3c	Acidic alumina	MW	30	70	76–78[e]
	Toluene	MW	15	82	
	Toluene	Δ	360	19[c]	

MW, Microwave activation; Δ, Classical heating.
[a] Final temperature evaluated by IR detection for microwave methods, by digital thermometer for classical methods.
[b] Yield refers to pure isolated product.
[c] Complement = starting materials + degradation compounds.
[d] Complement = starting materials.
[e] From EtOH 70%.

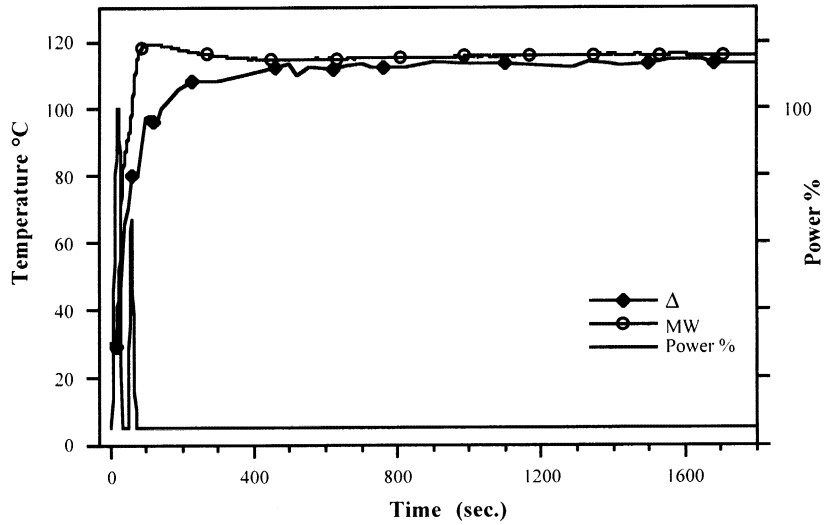


Fig. 1. Profiles of the rise in temperature by classical heating (◆) and microwave irradiation (○) for the synthesis of compound **2b**.

2.7.1. Treatments of the keratinocyte cultures

Cells were seeded 24 h before treatment in 96-well plates at 4×10^4 cells/well, in order to obtain semi-confluent cultures. Cells were exposed for 3 h to the medium containing various concentrations (from 0.025 to 70 mM) of compounds **2a**, **2c** and **3b,** dissolved in 1% dimethylsulphoxide (final concentration, DMSO). Positive controls that received sodium dodecylsulphate (SDS), and controls for solvent were carried on. After that time the medium was removed and the plates were washed twice with phosphate-buffered saline (PBS) to be prepared for the NRU and MTT assays.

2.7.2. Neutral red uptake

Cells were exposed to a medium containing 50 μg/ml of neutral red dye and processed according to the method of Borenfreund et al. (1998). After incubation for 3 h at 37 °C, the cells were washed twice in PBS and fixed according to the procedure described by Riddell et al. (1986). Then the plates were left at room temperature for 10 min and the absorbance of the extracted neutral red dye was read at 550 nm wavelength in an Uniskan II microplate reader (Labsystems, Helsinki, Finland).

2.7.3. MTT (tetrazolium) assay

MTT was dissolved in PBS (5 mg/ml) and given to the cells (10 μl/ml medium), according to the method of Mosmann (1983). After incubation for 3 h at 37 °C, a solution of hydrogen chloride 1 N/isopropanol (1:24 v/v) was added to each well and mixed to dissolve the formazan dark blue crystals. After a few minutes at room temperature, the plates were read at 550 nm wavelength.

2.7.4. Statistical analysis

All the experiments were performed at least four times using four wells for each concentration of the agents tested. Results were expressed as a percentage of viability compared to control.

Linear regression analysis was used to compute the concentration needed to reduce absorbance to 50% (IC_{50}).

2.8. Antimicrobial activity

The following test microorganisms were chosen as a target for the microbicidal activity evaluation: *P. aeruginosa*, *E. coli*, *S. hominis*, *C. albicans*, *P. acnes.*

They were cultured in Tryptone soya agar (TSA), Tryptone soya broth (TSB), fluid thioglycollate medium, sulphite polimixin sulphadiazine

Table 4
Odour evaluation of compounds **2a**, **2c**, **3a**, **3b**

Sample **2a**	
Note	Synthetic note, similar to a combination of camphor and menthol
Reference note	Menthol, camphor
Starting intensity	Not very diffusive
Evolution	The note evolves towards an aliphatic solvent-like note, or like *p*-dichlorobenzene
Persistence	Good
Persistence after 24 h	Low
Use	Interesting top note for pharmaceutical and cosmetic products (shampoos containing tars)
Sample **2c**	
Note	Weak ethereal, minty, woody
Reference note	Mentha aquatica
Starting intensity	Low
Evolution	The note is constant, with a low diffusion
Persistence	Low, fresh sensation
Persistence after 24 h	Weak as a pure product, not perceptible in solution
Use	Fresh note interesting for flavouring toothpaste for its fresh persistent characteristics
Sample **3a**	
Note	Menthol with a green undertone, oregano
Reference note	Origanum oil
Starting intensity	Weak
Evolution	The note evolves towards a woody note (cedarwood), with an increasing oregano note
Persistence	Good
Persistence after 24 h	Medium, the oregano note is still perceptible, but weaker
Use	Interesting note in perfumery, for its accordance of menthol and oregano, very natural and clean. Useful as top and middle note with herbaceous and green characteristics
Sample **3b**	
Note	Pleasant menthol-like note
Reference note	Menthol, isomenthone
Starting intensity	Weak, diffusive
Evolution	The note evolves towards a woody note, with dry woody characteristics (cedramber)
Persistence	Good
Persistence after 24 h	Weak but it is still perceptible a menthol-like note
Use	Interesting note in perfumery as middle and top note, for its peculiar fresh and balsamic nuances. Useful in toothpaste aromas

(SPS) agar, after dilution in Tryptone water dilution (TWD). The antimicrobial activity was determined by the Reybrouck-in vitro quantitative suspension test (Reybrouck, 1979), which was used to evaluate the killing effect of the two selected compounds, **2c** and **3b**, against the five test microorganisms after 5 min of exposure. The compounds were diluted at the following concentration: 0.1–0.2–0.3 mmol/ml in tetraglycol.

2.8.1. Treatment of the microbial suspensions

The bacterial strains were obtained from the third subculture at 37 ± 1 °C/24 h on TSA slants from freeze-dried stock cultures. The bacterial suspensions were obtained in TSB after an incubation for 24 h at 37 ± 1 °C, followed by centrifugation at 2000 rpm for 20 min and resuspension in TWD. The number of viable organisms in the inoculum was determined by the plating technique, by mixing 1.0 ml aliquots from the 10^{-6}, 10^{-7} and 10^{-8} dilutions with 20 ml of TSA. All the inoculations were carried out in triplicate. The test was carried out in a water bath at 25 ± 1 °C. For *P. acnes* SPS agar and FTM were used in anaerobic conditions (Anaerobic Plus System, Oxoid/Unipath, Milan, Italy). At zero time 1 ml of the chemical compound solutions was added to 0.1 ml of each bacterial suspension. After 5 min, 9 ml of LPHT inactivator solutions (0.3% lecithin, 3% polysorbate 80, 0.1% histidine, 0.5% sodium thiosulphate) were added to the medication mixtures. After a 30 min contact time, 0.1 ml of the undiluted inactivated mixture and of the 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions in TWD were spread in triplicate on plates containing 20 ml of TSA. The colony forming units were counted after incubation at 37 ± 1 °C for 24 h. For the negative controls, which were submitted to the same medication procedure, TWD without the test-chemical compound was used. The germicidal activity was numerically expressed using the Germicidal Effect ($G.E. = \log Nc - \log Ns$; the decimal reduction time) which is the ratio, expressed as logarithm, between the number of colony forming units (CFU) per ml of the control unexposed mixture (Nc) and the exposed one (Ns).

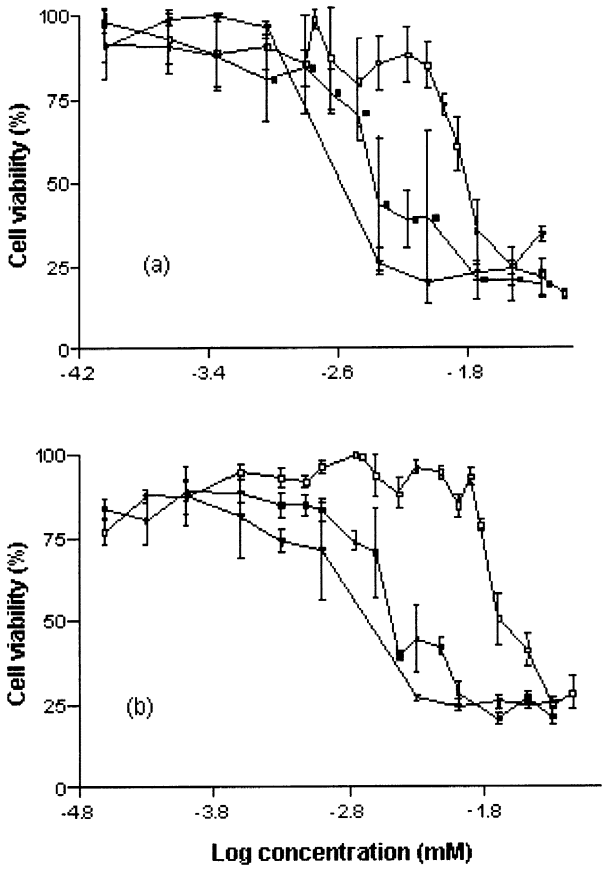


Fig. 2. Comparative cytotoxicity of compounds **2a** (□), **2c** (■) and **3b** (▼) in NCTC 2544 human keratinocytes as detected with: (a) NRU; (b) MTT assays. The values represent the mean ± SEM of four experiments in quadruplicate.

The corresponding killing effect percentage (K.E.%) was obtained by rating to 100 the difference between the CFUs of the negative controls and those corresponding to the microorganisms which survived in the neutralized medication mixture.

3. Results and discussion

Microwave activation coupled with acidic catalysis in a 'dry media' (acidic alumina impregnated with 7% PTSA) appeared as an efficient, economical and eco-friendly method to obtain terpenoidic cyclic ketals. The reaction conditions and the yields are reported in Table 2 and Table 3.

The results, compared to those obtained by the classical method, showed a very important improvement in the chemical yields. The reactions carried out under microwave heating in toluene and PTSA as catalyst, lead to quite the same results compared to those in 'dry media'. Taking into account our interest in environmentally friendly processes, the solvent-free method should be considered the best.

A very strong microwave specific effect was evidenced when the syntheses of compounds **2b**, **2c** and **3b** were carried out under conventional heating, every condition equal to the microwave activation method (yields MW/Δ: 78/25 for **2b**, 75/15 for **2c**, 60/19 for **3b**). Such a conclusion was supported by the examination of quite identical profiles in rising temperatures which are reported, as an example, for compound **2b** (Fig. 1). For this compound, under conventional heating, the reaction time was also extended to 5 h (300 min): yields were similar (27 vs. 25%) and some degradation compounds were noted.

Four compounds (**2a**, **2c**, **3a**, **3b**) showed potentially interesting olfactive features for cosmetic use. Their odour properties are reported in Table 4. Compound **2a** presents a peculiar odour useful for anti-dandruff shampoos and compound **3a** is interesting as a note in perfumery. Compounds **2c** and **3b** exhibit peculiar fresh notes interesting as aromas for flavouring toothpastes.

The results obtained from the evaluation of the cytotoxicity of compounds **2a**, **2c** and **3b**, as done by means of in vitro tests, are shown in Fig. 2.

The dose–response curves show very similar patterns for both tests, being the differences among the three molecules particularly evident at concentrations greater than 0.5–1 mM. It is clear that, at the highest concentrations, **2a** is much less cytotoxic than **2c** and **3b**.

By comparing the IC_{50}s (the concentration killing 50% of the cell population), as shown in Table 5, it is clear that **2a** has an intrinsic cytotoxicity lower than **2c** and **3b**, since these latter compounds display IC_{50}s about 5–7 times lower than the former. However, all the molecules show lower IC_{50}s, of one order of magnitude, in comparison to a strong irritant compound: the positive control sodium dodecylsulphate (SDS) (Table 5).

Table 5
Cytotoxicity evaluation of compounds **2a**, **2c**, **3b** and sodium dodecylsulphate (SDS) in NCTC 2544 human keratinocytes evaluated as IC50 (the dose inhibiting viability to 50%)

Compound	IC 50 (mM)	
	NRU test	MTT test
2a	14.79 ± 1.205	21.90 ± 1.267
2c	3.64 ± 1.163	2.43 ± 1.183
3b	2.36 ± 1.515	0.96 ± 0.524
SDS	0.13 ± 0.002	0.13 ± 0.001

The results of the preliminary antimicrobial activity tests obtained for compounds **2c** and **3b** and reported on Table 6 and Fig. 3, Fig. 4, revealed a mild germicidal activity which was generally proportional to the concentrations used, except in the case of *C. albicans*.

The lowest bactericidal activity was found with *P. acnes* (G.E. values between 1.234 and 2.362 for compound **2c** and between 1.478 and 1.738 for compound **3b**).

This means that both compounds **2c** and **3b** could also be used in cosmetics at the lowest concentration of 0.1 mmol/ml having shown a Germicidal Effect over 3.1 Log Units.

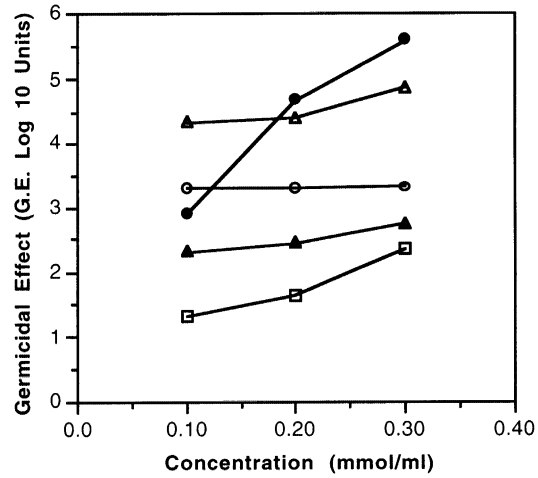


Fig. 3. Germicidal effect of compound **2c** vs. *Ps. aeruginosa* (▲), *E. coli* (●), *St. hominis* (△), *C. albicans* (○), *P. acnes* (□).

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Table 6
Germicidal Effect (G.E) and Killing Effect percentage (K.E.%) of compounds **2c** and **3b** at the concentration of 0.1, 0.2 and 0.3 mmol/ml in tetraglycol

Compound	Concentration (mmol/ml)		*Ps. aeruginosa*	*E. coli*	*St. hominis*	*C. albicans*	*P. acnes*
2c	0.1	G.E.	2.340	2.913	4.327	3.294	1.324
		K.E.%	99.543	99.878	99.995	99.949	95.262
	0.2	G.E.	2.470	4.687	4.396	3.306	1.647
		K.E.%	99.661	99.998	99.996	99.951	97.744
	0.3	G.E.	2.758	5.594	4.873	3.325	2.362
		K.E.%	99.826	99.999	99.999	99.953	99.565
3b	0.1	G.E.	2.302	3.452	1.805	3.180	1.478
		K.E.%	99.501	99.965	98.434	99.934	96.677
	0.2	G.E.	2.303	3.613	2.310	3.191	1.682
		K.E.%	99.502	99.976	99.510	99.936	97.920
	0.3	G.E.	2.324	3.793	2.994	3.192	1.738
		K.E.%	99.525	99.984	99.899	99.936	98.170

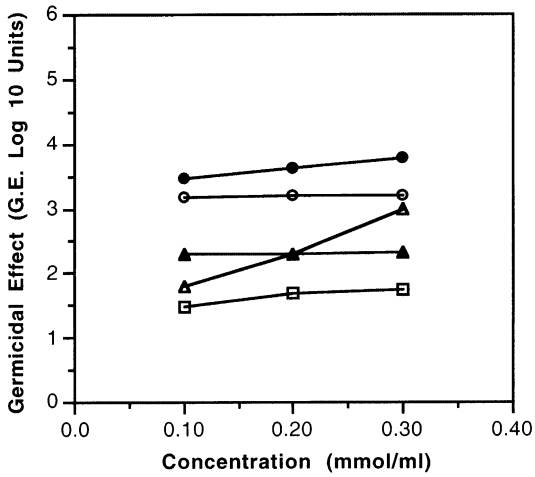

Fig. 4. Germicidal effect of compound **3b** vs. *Ps. aeruginosa* (▲), *E. coli* (●), *St. hominis* (△), *C. albicans* (○), *P. acnes* (□).

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