Stimulation of humoral immunity in mice by undecan-2-one, undecan-2-ol and their derivatives

JULIA GIBKA [1], EWA SKOPIŃSKA-RÓŻEWSKA [2], ANDRZEJ K. SIWICKI [3], ALEKSANDER WASIUTYŃSKI [2], EWA SOMMER [2], JANUSZ BANY [4]

[1]Institute of General Food Chemistry, Technical University of Lodz; [2]Department of Pathology, Biostructure Center, Medical University of Warsaw; [3]Department of Microbiology and Clinical Immunology, Warmian-Mazurian University, Olsztyn; [4]Department of Toxicology and Pharmacology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

Abstract

The in vivo effect of some derivatives of aliphatic 2-ketone, undecan –2-one, on humoral immunity in mice were studied. Stimulatory effect on anti-SRBC antibody production was presented by undecan-2-ol, undecan-2-olR(-) enantiomer, undecan-2-ol acetate and undecan-2-one acetals. No effect of S(+) enantiomer of undecan-2-ol was observed. Serum lysozyme level increased in mice inhaled with undecan-2-ol.

Key words: *undecan-2-ol, enantiomers, humoral immunity, mice.*

(Centr Eur J Immunol 2008; 33 (2): 47-49)

Introduction

Undecan-2-one is a component of a few essential oils and the extracts obtained from many exotic plants. High concentrations of it are observed in the essential oils derived from plants belonging to the *Rutaceae* family. The oil from *Ruta chalepensis* L. which grows in Turkey was reported to contain undecan-2-one (66.5%) and that from *R. montana* up to 84.2% of this ketone [1]. *Ruta graveolens* which grows in China contains 67.0% of undecan-2-one [2] and that from Cuba 48.7% [3]. *Siparuna guianensis* (Aubl.) from the Brazilian Amazonian Region was found to possess undecan-2-one in 32.5% and 31.7% in the fruit oil and leaf oil, respectively [4]. The ketone has also been found in the oils derived from *Zanthoxylum pinnatum* and *Cymbopogon schoenanthus* (L.) Spreng (54.3 and 14.7%, respectively) [5, 6]. High contents of this ketone have also been observed in the bark oil from *Glycosmis pentaphylla* Cor. (58.1%) [7] and in the root oils from *Pilocarpus spicatus* St Hill. (20.8%) [8] and *Philodendron acutatum* Schott. (9.6-12.7%) [9].

The synthetic undecan-2-one is often used in the food and fragrance industry, owing to its pleasant fruit – floral odour with an orange – herbal note.

Recently, we have reported some data on the immunotropic effects of undecan-2 one [10]. This substance, introduced to mice by inhalation increased their cellular and humoral immunity in various *in vivo* and *ex vivo* tests. The aim of the present study was to evaluate the effect of its derivative, undecan-2-ol, and various derivatives of undecan--2-one and undecan-2-ol on humoral immunity in mice.

Materials and Methods

Synthesis of ketone

Undecan-2-one was prepared by dr Marek Gliński in Faculty of Chemistry, Warsaw Technical University, by ketonization of a mixture of acetic and decanoic acids in the presence of 20wt%MnO_2/Al_2O_3 catalyst under flowing conditions. Yield of ketone 70.0%, purity 99.9% (GC), n_D^{20}=1,4262. The purity of the ketone, physical properties and spectral data were determined [11].

Synthesis of alcohol

2-undecanol was prepared by reduction of 2-undecanone using sodium borohydride in a water/propan-2-ol solution. Yield of undecan-2-ol 91%, its purity 98.9% (GC)

Correspondence: Ewa Skopińska-Różewska, Pathology Department, Biostructure Center, Warsaw Medical University, Chałubińskiego 5, 02-004 Warsaw, Poland, Email: ewaskop@hotmail.com

[12]. It has a fatty with a mild fruity note odour and therefore can be used as potential starting material for the creation of perfumes and flavours for the corresponding cosmetics and food industries.

Ethylene and propylene acetals of undecan-2-one were prepared starting from undecan-2-one.

Synthesis of acetals

Acetals were prepared in the reaction between ketone and ethane-1, 2-diol or propane-1,2-diol in toluene in the presence of sulfosalicylic acid as a catalyst. All derivatives were prepared in a 2-5 g scale [13].

Synthesis of enantiomers

Enantiomerically pure undecan-2-ols and undecan-2-ol acetates were prepared by biocatalytic enantioselective synthesis. Products of these reactions were separated on the column chromatography. Column chromatography was performed on silica gel (Kieselgel 230-400 mesh) and mixture of hexan, etyl acetate. Specific rotations of enantiomers were measured in etanol with an Autopol (Rudolf) polarimeter.

The absolute configuration of enantiomers was determined by correlation with literature data [14, 15]. The purity of compounds was confirmed by GC/MS, GC (Chirasil-Dex column), IR, ^{1}H NMR and the measurements of refractive index.

The ^{1}H NMR spectra were recorded on a Bruker 250 DPX spectrometer, in $CDCl_3$ using TMS as internal standard. The IR spectra were measured using Specord M 80 spectrometer (film or KBr pellet). Microanalyses were performed on a Perkin Elmer 2400 CHN elemental analyser. Melting points (uncorrected) were determined on Boetius apparatus.

Mice

The study was performed on 10-12 weeks old female Balb/c and (Balb/cxDBA2)F1 mice, weighing 25-28 g, delivered from the Polish Academy of Sciences and from the own breeding colonies.

Study of antibody production

Mice were immunized with 10% SRBC (0.1 ml intraperitoneally), and subjected to inhalations for 3 consecutive days, according to the following scheme: 5 mice in one cage, 5 drops of tested compound for 60 minutes, the cage covered by linen during inhalation. The cages with control mice were accordingly covered by linen for 60 minutes.

Mice were bled in anaesthesia (3.6% chloral hydrate), from retroorbital plexus, 7 days after immunization. The antibody level was evaluated with haemagglutination assay in inactivated (56°C, 30 min) sera. After performing a series of sera dilutions, 0.5 % SRBC were added and the mixture was incubated for 60 min at room temperature, then centrifuged (10’, 150 g) and shaken. The hemagglutination titer was evaluated in a light microscope – as the last dilution in which at least 3 cell conglomerates were present in at least 3 consecutive fields at objective magnification 20×. Stimulation indices were calculated as a ratio of log titer of each experimental serum to the mean log titer of simultaneously performed control.

Lysozyme level was determined according to the method described [10]. Statistical analysis was performed by Student *t*-test. Experiments were approved by the Local Ethical Committee.

Results

Lysozyme concentration in the sera of mice inhaled for 3 days with undecan-2-ol was significantly higher than in the sera of control ones (9.80±0.40 mg/L versus 7.85±0.45 mg/L, $P<0.01$).

The effect of undecan-2-one, undecan-2-ol and their derivatives on antibody production is presented on the Table 1.

Table 1. The effect of undecan-2-one and its derivatives on antibody production in mice

Compound used for inhalation	Number of mice	Mean stimulation index of antibody titer ±SE	Significance of difference from the control
Controls (non-inhaled)	40	1±0.04	–
Undecan-2-one 10%	5	1.38±0.02	P<0.001
Undecan-2-ol 1%	5	1.37±0.03	P<0.001
Undecan-2-ol 10%	5	1.29±0.02	P<0.001
S(+) undecan-2-ol (E-1) 1%	10	1.04±0.13	n.s.
R(–) undecan-2-ol (E-2) 1%	10	1.36±0.06	P<0.01
S(+) undecan-2-ol acetate(E-3) 1%	10	1.31±0.05	P<0.01
R(–) uncecan-2-ol acetate(E-4) 1%	10	1.34±0.11	P<0.01
Undecan-2-one ethylene acetal 1%	10	1.64±0.06	P<0.001
Undecan-2-one propylene acetal 1%	10	1.72±0.07	P<0.001

Except S(+) which was one of the two enantiomers of undecan-2-ol , all other tested substances highly significantly stimulated this parameter of immunity.

Discussion

In this paper we present for the first time the evidence of immunostimulatory activity of racemic undecan-2-ol and its component, pure R(–) enantiomer, as well as of some other derivatives (acetate and acetals). Similarly to undecan 2-one, undecan-2-ol has been found in nature too. It exists in many plants, as one of the major components of essential oils from *Ruta graveolens* L. [3] and *Philodendron acutatum* Schott. [9] and from *Eucalyptus risdoni*, from *Curcuma aeruginosa* Roxb. and *Curcuma heyneana* Val. [16].

R(–) – undecan-2-ol was detected in the essential oil from *Listea odorifera*, S(+) undecan-2-ol has been found in cacao butter [17]. *Ruta graveolens* is also a natural, rich source of undec-2-yl acetate [2].

Ethylene acetal of undecan-2-one and propylene acetal of undecan-2-one are newly prepared compounds. Furthermore, besides undecan-2-on is present in many plants, ethylene and propylene acetals of undecan-2-one have not been found in nature. They have characteristic pleasant odours and therefore can be used as potential starting materials for the creation of perfumes and flavours for the corresponding cosmetics and food industries. Ethylene acetal has a intensive, herbaceous-spicy odour, propylene acetal has a nettle-like with a pitch note odour [13].

Immunostimulatory fragrances and food additives may be beneficial for improving immune system function in immunocompromised subjects, and for additional, immunostimulatory treatment of patients suffering from various infections. However, abuse of foods and fragrances with immunomodulatory properties may be not recommended for healthy persons everyday use [18].

Acknowledgment

Financial support from the Polish State Committee for Scientific Research (Grant KBN No. 3T09B 062 28) is gratefully acknowledged.

References

1. Baser KH, Özek T, Beis SH (1996): Constituents of the essential oil of Ruta chalepensis L. from Turkey. J Essen Oil Res 8: 413-414.
2. Lawrence BM, Reynolds RJ (1998): Progress in Essential Oils. Perfumer & Flavorist 23: 50.
3. Pino J, Rosado L, Fuentes V (1997): Leaf oil of Ruta graveolens L. grown in Cuba. J Essent Oil Res 9: 365-366.
4. Fischer DC, Limberger RP, Henriques AT, Moreno PR (2005): Essential oils from fruits and leaves of Siparuna quianensis (Ablu.) Tulasne from Southeastern Brazil. J Essent Oil Res 17: 101-102.
5. Brophy JJ, Goldsack RJ, Forster P, Hutton I (2000): Composition of the Leaf of the Australia and Lord Howe Island Species of Zanthoxylum (Rutaceae). J Essent Oil Res 12: 285-291.
6. Shahi AK, Tava A (1993): Essential Oil Composition of Three Cymbopogon Species of Indian Thar Desert. J Essent Oil Res 5: 639-643.
7. Ahmed R, Choudhury S, Vajczikova I, Leclercq PA (2000): Essential oils of Glycosmis pentaphylla (Cor.). A new report from Assam India. J Essent Oil Res 12: 471-474.
8. Neto MA, Cunha UA, Mafezoli J, Silveira ER (2002): Volatile constituents of different populations of Pilocarpus spicatus Saint Hill. (Rutaceae) from the Northeast of Brazil. J Essent Oil Res 14: 319-324.
9. Viana F, Andrade-Neto M, Pouliquen YB, Lucie VG (2002): Chemical Composition of the Essential Oil from Roots of Philodendron acutatum Schott. J Essent Oil Res 14: 172-174.
10. Skopińska-Różewska E, Gibka J, Gliński M et al. (2006): Immunotropic effects of undecan-2 one in mice. Centr Eur J Immunol 31: 57-62.
11. Gliński M, Gibka J (2004): Catalytic ketonization over oxide catalyst. Part IX. Single step synthesis of aliphatic saturated and unsaturated C_{11} – C_{13} ketones from carboxylic acids. Polish J Chem 78: 299-302.
12. Gibka J, Gliński M (2006): Derivatives of undecan-x-ones (x=2–6): Synthesis and Spectral Data. Zeszyty Naukowe PŁ, Chemia Spożywcza i Biotechnologia 70: 5-12.
13. Gibka J, Gliński M: Olfactory properties of straight-chain undecan-x-ones, undecan-x-ols (x=2–5) and their derivatives. Flavour Fragr, in press.
14. Nakamura K, Matsuda T (1998): Asymmetric reduction of ketones by the acetone powder of Geotrichum candidum. J Org Chem 63: 8957-8964.
15. Dangel BD, Polt R (2000): Catalysis by amino acid-derived tetracoordinate complexes: Enantioselective addition of dialkylizincs to aliphatic and aromatic aldehydes. Org Lett 2: 3003-3006.
16. Burdock GA: Handbook of flavor ingredients. CRC Press, Boca Raton 2002, 1758.
17. Djerassi C: Dictionary of Natural Products. Chapman & Hall, New York 1994, U-00060.
18. Gibka J, Skopińska-Różewska E, Siwicki AK et al.: Działanie immunotropowe i wpływ na wzrost doświadczalnego mięsaka u myszy undekan-x-onów, undekan-x-oli i ich pochodnych (x=2–4). In: Skopińska-Różewska E, Siwicki AK (eds.). Endogenne i egzogenne modulatory odporności i angiogenezy. SPW EDYCJA, Olsztyn 2007, 61-78.