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(54) **N-ALKYLCARBONYL-AMINO ACID ESTER AND N-ALKYLCARBONYL-AMINO LACTONE COMPOUNDS AND THEIR USE**

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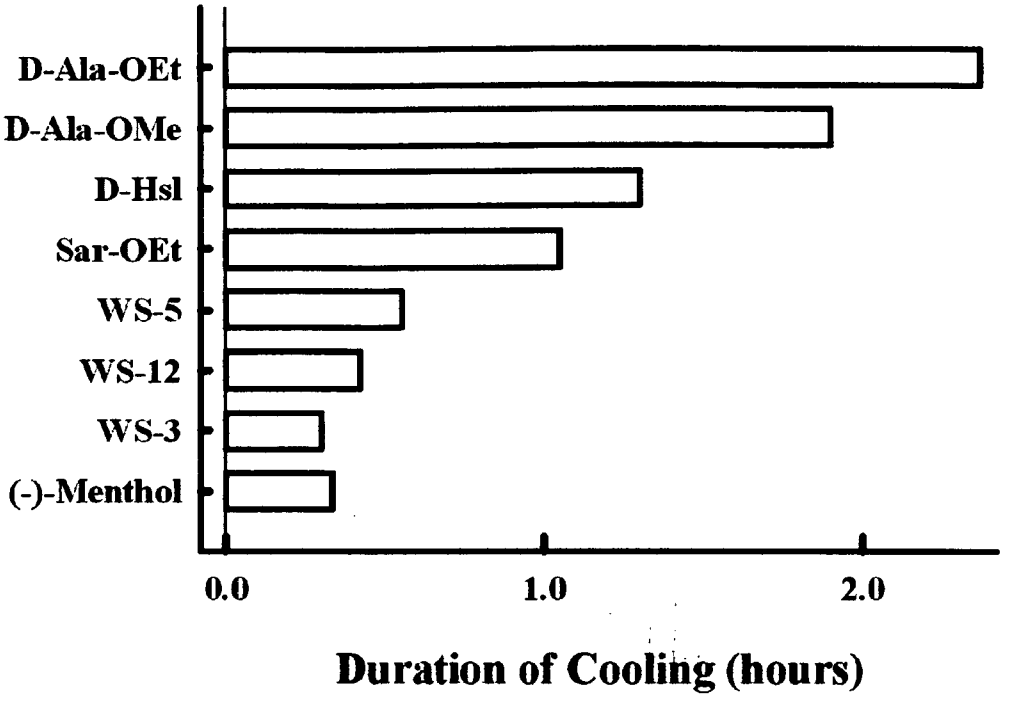
(57) **ABSTRACT**

The present invention generally relates to refreshing, soothing, and cooling compounds that affect sensory processes. More particularly, the present invention pertains to certain N-alkylcarbonyl-amino acid ester as described herein; compositions and articles comprising such compounds; and methods of treatment, for example, methods of increasing alertness, and decreasing fatigue or sleepiness, and alleviating ocular discomforts of irritation, itch, and pain.

FIGURE 1


D-Ala-OEt
D-Ala-OMe
D-Hsl
Sar-OEt
WS-5
WS-12
WS-3
(-)-Menthol
0.0
1.0
2.0
Duration of Cooling (hours)

N-ALKYLCARBONYL-AMINO ACID ESTER AND N-ALKYLCARBONYL-AMINO LACTONE COMPOUNDS AND THEIR USE

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application 20080227857, filed Mar. 23, 2006, inventor Wei, entitled "N-Alkylcarbonyl-Amino Acid Ester and N-Alkylcarbonyl-Amino Lactone Compounds and Their Use", incorporated by reference.

[0002] This application is related to:

[0003] U.S. Provisional Application No. 60/667,166 filed 29 Mar. 2005;

[0004] U.S. Provisional Application No. 60/683,384 filed 20 May 2005;

[0005] U.S. Provisional Application No. 60/702,505 filed 26 Jul. 2005;

[0006] U.S. patent application Ser. No. 11/203,728 filed 13 Aug. 2005; and

[0007] U.S. Provisional Application No. 60/772,374 filed 9 Feb. 2006;

[0008] the contents of each of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0009] The present invention generally relates to refreshing, soothing, and cooling compounds that affect sensory processes. More particularly, the present invention pertains to certain N-alkylcarbonyl-amino acid ester and N-alkylcarbonyl-amino lactone compounds; compositions and articles comprising such compounds; and methods of treatment, for example, methods of alleviating the discomforts of irritation, itch, and pain on the skin and on the ocular surface.

BACKGROUND

[0010] Menthol and menthol-like compounds are used in toiletries, confectionery, comestibles, and over-the-counter medications as ingredients to refresh, cool, flavor, counter-irritate, and anesthetize the skin and mucous membranes of the mouth and upper airways. Menthol's utility in relief of sensory discomfort is, however, limited by its short duration of action and by its multimodal actions on sensory processes—including odor, harshness of taste, and irritation. The unpleasant effects of menthol can be easily experienced, for example, when menthol-containing ointments are brought near the eye surface. The menthol vapors hurt the eye and causes tearing.

[0011] There is a need for compounds like menthol that refresh, cool, and soothe the body's surfaces, but without the disadvantages of odor, irritancy, and most importantly, a short duration of action. It is important to have compounds that act much longer than menthol and without irritation.

[0012] About three decades ago, a group of scientists synthesized over 1200 compounds in an attempt to find cooling agents that had properties better than menthol. Their results were summarized in a paper (Watson et al, "New compounds with the menthol cooling effect," J. Soc. Cosmet. Chem., 29: 185-200, 1978). From this research, an N-alkyl-cycloalkyl- and an N-alkyl-alkyl carboxamide, WS-3 and WS-23, were brought to the market and are used as additives for confectionery, comestibles, (e.g., candy, chewing gum), and toiletries. In U.S. Pat. No. 4,178,459 (11 Dec. 1979), Watson et al. described cooling properties of some N-alkoxycarbonylalkyl-substituted p-menthane-carboxamides.

[0013] None of the compounds currently known to the art have the potency or duration of action to qualify them as possible medications for use in disorders of eye discomfort such as itch (allergic conjunctivitis), pain, and dryness.

SUMMARY OF THE INVENTION

[0014] One aspect of the present invention pertains to certain N-alkylcarbonyl-amino acid ester compounds, as described herein.

[0015] Another aspect of the invention pertains to a composition comprising such a compound and a delivery vehicle (e.g., for delivering the compound to a human).

[0016] In one embodiment, the delivery vehicle is a towelette.

[0017] In one embodiment, the compound is present in the composition in an amount of 0.02 to 0.2% wt/vol (0.2 to 2 mg/ml).

[0018] Another aspect of the present invention pertains to use of such a compound in the manufacture of a medicament for use in a method of treatment.

[0019] In one embodiment, the treatment is the relief of (e.g., alleviation of) ocular irritation, itch, and/or pain (e.g., wherein the contacting delivers an amount of the compound that is therapeutically effective for alleviation of irritation, itch, and/or pain).

[0020] In one embodiment, the treatment is treatment to increase alertness, or to decrease sleepiness and fatigue (e.g., wherein the contacting delivers an amount of the compound that is effective to increase alertness, or to decrease sleepiness and fatigue).

[0021] As will be appreciated by one of skill in the art, features and preferred embodiments of one aspect of the invention will also pertain to other aspect of the invention.

[0022] Other advantages and aspects of the invention will be understood by reading the following detailed description and the accompanying claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. **1** is a bar graph showing duration of cooling (hours) for eight compounds (in order): D-Ala-OEt, D-Ala-OMe, D-Hsl, Sar-OEt, WS-5, WS-12, WS-3, and (−)-menthol.

DETAILED DESCRIPTION

[0024] A class of compounds that is suitable to be used as an active ingredient in (e.g., pharmaceutical) preparations for use on the skin of the eyelids and on the ocular surface has been found.

[0025] These compounds are suitable, for example, for use as agents to reduce discomfort such as itch, a sense of dryness and irritation, and pain.

[0026] These compounds have one or more of the following properties:

[0027] (i) a refreshing, soothing, and cooling action on the surface of the skin and, in pathological states, act as an anti-irritant, anti-pruritic, and/or anti-nociceptive agent

[0028] (ii) a minimal irritant action on the eye when the compound is applied to facial skin near and on the ocular surface;

[0029] (iii) a rapid onset of action of less than 5 minutes, preferably less than 1 minute;

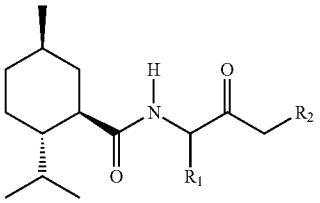
[0030] (iv) a duration of action that exceeds 1 hour, for example, when applied onto the upper eyelids at a concentration of 0.2 to 2 mg/ml in a towelette; and

[0031] (v) a robust cool, soothing, and refreshing sensation when applied that counteracts noxious or irritating stimuli on the ocular surface and the mucous membranes of the eye.

[0032] These compounds may conveniently be referred to as N-alkylcarbonyl- and N-alkyl-N-alkylcarbonyl-D-, L-, or DL-amino acid esters or "NACE compounds".

[0033] In one embodiment, the compound is selected from compounds of Formula (1):

Formula (1)



[0034] wherein:

[0035] R_1 is hydrogen, or a methyl group such that the carbon atom to which it is attached is in the D-configuration, and

[0036] when R_1 is hydrogen, R_2 is isopropyl, and

[0037] when R_1 is a methyl group such that the carbon atom to which it is attached is in the D-configuration, R_2 is a C_1 to C_3 alkyl group.

[0038] The menthyl group (i.e., the 5-methyl-2-(1-methylethyl)cyclohex-1-yl group) has the same stereochemistry as found in (−)-menthol.

[0039] The α-carbon, between the —NH— group and the —C(═O)OR_2 group, has the same stereochemistry as found in D-alanine. This is also known as the R-configuration, according to the Cahn-Ingold-Prelog nomenclature system. Except for glycine, all α-amino acids have a chiral center at the α-carbon. Although amino acids of the D-configuration are found in some antibiotics and in cell membranes of microorganisms, the amino acids of proteins are (almost) exclusively of the L (or S configuration).

[0040] In one embodiment, R_2 is independently methyl, ethyl, or isopropyl

[0041] The isopropyl ester and the D-configuration have the effect of increasing potency and duration of action, and of producing a selective refreshing coolness near or on the ocular surface with the absence of tissue irritation.

[0042] Examples of some preferred compounds are shown in Table 1, ranked according to the duration of action on the ocular surface:

TABLE 1

Preferred embodiments for applications.

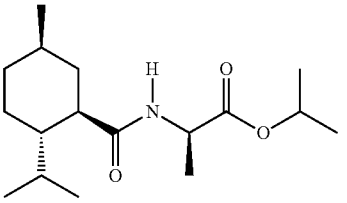
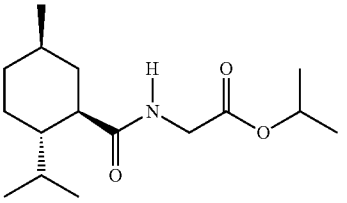
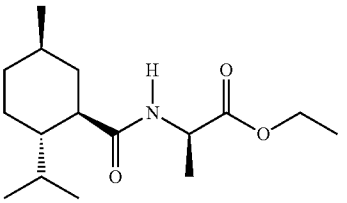
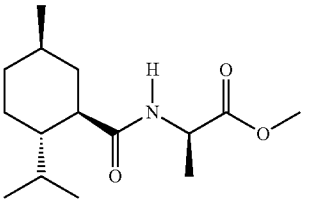
Code	Chemical Name	Structure
1 D-Ala-OiPr	(R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid isopropyl ester	
2 Gly-OiPr	(R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-acetic acid isopropyl ester	
3 D-Ala-OEt	(R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid ethyl ester	

TABLE 1-continued

Preferred embodiments for applications.

Code	Chemical Name	Structure
4 D-Ala-OMe	(R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid methyl ester	

[0043] Preferred among these NACE compounds are "long-acting" NACE compounds that, when applied to the skin or mucous membranes, produce refreshing, soothing, and cooling sensations without skin irritation, with minimal eye irritation, and with a duration of action on skin that lasts more than about 1 hour when used at 0.20 to 2.0 mg/ml or less.

[0044] The long-acting NACE compounds are distinguished from other N-alkylcarbonyl-amino acid esters (e.g., WS-5, see FIG. **1**) and N-alkyl substituted carboxamides (e.g., WS-3, WS-12, WS-23; see FIG. **1**) (WS-23 is N-2,3-trimethyl-2-isopropylbutanamide), which are known to have cooling properties and the two (WS-3 and WS-23) that are commercially used in comestibles, confectionery, and toiletries.

[0045] As shown in Study 1, WS-3, WS-5, WS-12, WS-23 and WS-31, have a short duration of action (less than 1 hour) on the philtrum skin or slow onset (more than 5 minutes). Also, some of these compounds do not achieve significant cooling but rather produce skin sensations of tingling, burning, and irritation, effects similar to those observed with (−)-menthol, a compound with multimodal actions of sensory processes.

[0046] By contrast, the preferred long-acting NACE compounds deliver a perfect cooling sensation, with rapid onset, long duration of action, and minimal skin or eye irritation that has not been previously recognized.

[0047] Furthermore, the potency, duration, and selectivity (absence of irritation) of action are increased for the compounds of Formula 1, with R_1═H, or C wherein the α-*carbon* is in the D-configuration, and R_2 is isopropyl.

[0048] Due to their prolonged activity, the compounds, compositions, and articles may be used to inhibit the perception of itch, pain, and discomfort from the skin and the mucous membranes of the eyelids and ocular surface.

[0049] These compounds are without odor, smarting, or burning sensations on the facial skin and in one embodiment, the compound is carried on a towelette adapted for, or of sufficient construction for, the delivery of a therapeutically effective dose.

[0050] The specific structural features of the molecules that confer the desired properties of increased potency and duration of action, and the presence of refreshing cooling without irritation were unexpected and surprising and not known in the prior art.

[0051] The term "effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

[0052] Pharmacology and Mechanisms of Action of N-Alkylcarbonyl-Amino Acid Esters

[0053] Noxious stimuli from the skin are thought to be transmitted by unmyelinated C fibers and thinly myelinated Aδ fibers, together functionally called polymodal fibres. Noxious stimuli produce sensory discomforts on the skin and in the mucous membranes, discomforts which are ameliorated by cooling (vide infra). Cooling of the facial skin and mucous membranes is detected by a subset of primary sensory afferents that have receptors on nerve endings. These sensory fibers exhibit a rhythmic, ongoing discharge at neutral temperatures that increases in response to skin temperature cooling (step reductions from 33 to 23° C.) and are suppressed by warming. The dynamic information is propagated along axons in spike trains, at about 20 to 40 impulses/sec, to central neurons, leading to cooling sensations. This type of sensation is mimicked by facial exposure to air or water temperatures of 15 to 22° C. The primary afferents from facial skin terminate in the superficial layer of the caudal trigeminal nucleus where they represent over 95% of the thermoceptive input (see, e.g., Hutchinson et al., J. Neurophysiol., 77:3252-3266, 1997).

[0054] The precise mechanisms underlying the benefits of refreshing cooling on sensory discomfort are not clearly understood, although such benefits are a common experience. Sensations can be "confusing" when a chemical affects more than one sensory modality. This is especially true for (−)-menthol (also known as L-menthol, (1 R)-menthol, and (1 R,2S,5R)-menthol). (−)-Menthol is widely used as a cooling agent but it has multimodal action on sensory processes. For example, in the upper airways and oral cavity, (−)-menthol can elicit cooling, irritation, tingling, minty flavor, and bitter taste. Especially around the eyes, menthol is an irritant and elicits sensations of burning, stinging, and pain.

[0055] The peripheral (−)-menthol coolness receptor is thought to be a protein called TRP-M8. However, it has been found that the potency of compounds that activate TRP-M8 is not correlated to cooling actions (see, e.g., A. K. Vogt-Eisele, D. Bura, H. Hatt, and E. T. Wei. N-Alkylcarboxamide Cooling Agents: Activities on Skin and Cells with TRPM8 and TRPA1 Receptors. 3rd Annual Workshop on the Study of Itch, Sep. 25 to 27, 2005 in Heidelberg, Germany. Acta Dermato-Venereological 85: pg.468, 2005). Furthermore, TRP-M8 is activated by mustard oil, an agent that produces the

pungent sensations of wasabi. Thus, the compounds of the present invention should not be viewed as solely acting via TRP-M8 receptors.

[0056] Although menthol preparations, such as confectionery, have some alerting effects on the central nervous system, menthol compositions cannot be applied to facial skin in effective concentrations to arouse because it causes eye irritation (stinging, smarting, tearing, and pain). Thus, identification of an agent that does not irritate the eye surfaces, but which can be applied to facial skin to refresh and to reduce skin irritation, itch and pain would have utility.

[0057] Ideally, to treat skin and eye discomfort, a compound must act for at least one hour and preferably longer, otherwise the patient would have to repeatedly apply the drug to obtain relief. For an anti-irritant, the ideal agent should have rapid onset of action, soothing effects, and the ability to relieve discomfort for an extended duration, for example, for several hours.

[0058] Non-Technical Description of Inventive Concept

[0059] It is believed that the long-acting NACE compounds described herein activate the transmission of cool neurons so that the brain perceives the ambient temperature at about 15 to 18° C. Activation of these neurons is like turning on a robust air-conditioner within a hot environment. This sensory band in normal individuals is felt as alerting, refreshing, and cool. This is referred to herein as the "perfect cool." The presence of the NACE compounds and the perfect cool, in pathological conditions, gates the passage of noxious signals into the spinal cord and/or brain. Hence, a soothing anti-nociceptive (anti-irritant, anti-pruritic and antinociceptive) effect is achieved with therapeutic benefit.

[0060] The inventor has identified molecules with potent and prolonged activation of the perfect cool. These molecules are qualitatively and quantitatively unlike (−)-menthol and WS-3 which act for less than 20 minutes.

[0061] The long-acting NACE compounds are active at single doses per eye of 1 to 50 μg or at concentrations of 10 mg/mL or less when applied topically to the facial skin including the eyelids. The long-acting NACE compounds also have a rapid onset of action (from about 0.5 to about 3 minutes). The onset and offset of action of these compounds was first revealed by testing on the philtrum skin of subjects and then subsequently by applying them onto the skin near the eyes or on the closed eyelids with a towelette.

[0062] Bioassays of N-Alkylcarbonyl-Amino Acid Esters

[0063] Psychic events such as refreshment, soothing, cooling, irritation, itch, and pain cannot be verbalized by animals (animals cannot say "it feels cold", "ouch", or that "it itches"). Hence, the sensory effects of chemicals in animals must be indirectly inferred. Receptor assays, based on cells transfected with the genes for proteins associated with thermosensation (e.g., TRP-M8, TRP-A1, TRP-V1) may be used as a model of sensory processes. The receptor assays yield quantitative data but give no information on onset and offset of action, or on the quality of human sensations evoked by the chemicals. Furthermore, potency as measured by the median effective concentrations (EC_{60}) in the receptor assays may not be correlated to anti-nociceptive or cooling actions. Thus, the best information on the pharmacological properties of chemicals is derived from direct tests on humans.

[0064] Watson et al. (U.S. Pat. No. 4,178,459) tested the properties of N-substituted p-menthane carboxamides. However, the branched chain alkylesters such as the isopropyl analogs described here in the Gly and D-Ala configuration of Formula 1 were never synthesized by Watson et al. Bioactivity of the Watson et at compounds were tested by putting filter paper (1×1 cm), impregnated with a known amount of compound, onto the dorsal surface of the tongue of the volunteer test subject. After 30 seconds, the subject was required to report presence or absence of a cooling effect. These data were reported as "Threshold,**82** g" and refer to the threshold amount of the test substance that produces cooling sensations upon application onto the tongue of a panel of human volunteers. The average threshold of (−)-menthol for 6 subjects was 0.25 μg, but there was a 100-fold variation in individual sensitivity. The potencies of coolness signals detected from the dorsal surface of the tongue are not correlated to skin sensations of coolness may be confounded by gustatory, olfactory, and other variables, as well as by dilution from saliva.

[0065] It has been found that, if the goal is to find a drug useful for topical application, the refreshing cooling and sensory properties of a long-acting NACE compound are best tested first by suspending or dissolving a test substance in an ointment (usually Aquaphor®, which is 41% petrolatum, and the rest mineral oil, ceresin and lanolin alcohol) and singly applying the ointment (40 to 70 mg) onto the skin surface using a plastic stick. A reliable place for topical application is the skin above the upper lip (above the vermilion border of the lips), on the philtrum, lateral to the philtrum until the nasolabial folds, and on the lower nostrils (subnasale). This part of the face is known to be densely innervated with cold receptors, second only to the surfaces of the eyeball and anogenitalia.

[0066] The intensity of the subjective skin sensation is rated as 0, 1, 2 or 3 with: 0 as no change; 1 as slight coolness, cold, or tingling; 2 as clear-cut signal of coolness, cold, or tingling; and 3 as robust cooling or cold. The intervals for recording sensations are 5 to 10 minutes, until two successive zeroes are obtained. The results (shown in FIG. **1**) are averaged values of 4 to 6 separate trials in the same individual. The data are plotted using SigmaPlot® (Systat Software, Point Richmond Calif.) and a smoothing function with a negative exponential was used for analysis and statistical fit of the results. Confirmatory trials of cooling action of the long-acting NACE compounds were obtained in 2 to 4 individuals but not quantified for some because of the large number of chemicals that were evaluated

[0067] The onset of drug action is taken as the time to reach 2 units of coolness intensity, and offset of drug action is the time when coolness intensity drops below 2, after previously surpassing 2 units. The duration of cooling action is defined as the offset time minus the onset time. An inactive compound is defined as one that does not exceed 2 units of cooling for 5 minutes after application. The quality of the sensation is noted, for example, as pure refreshing coolness, or if the sensation is accompanied by irritation (stinging or burning). The quality of the sensation is not rated for intensity.

[0068] The ointment was also applied to the periorbital skin (upper and lower eyelids and on skin adjacent to the lateral canthus) for tests of irritancy near the eyes, and the subject is asked if irritation is present or absent. The intensity of the eye sensation is not rated.

[0069] A second method of testing was to take a towelette saturated with a test solution of the cooling agent and to wipe the towelette over the dosed eyelids. The presence or absence

of cooling (or irritanty, if applicable) sensations was then recorded at 10 minute intervals until the cessation of coolness in two successive intervals.

[0070] Qualitative Aspects of Cool and Cold Intensity

[0071] The long-acting NACE compounds described herein are useful as a topical agent for the relief of skin discomfort, and mimic the effects of running cold water on injured skin. The "nominal" ambient skin surface temperature to mimic with a cooling agent is in the range of 15 to 22° C. The effect can also be simulated by putting a towel wet with cold water onto the face. The coolness of a wet towel will rapidly dissipate, an effect called adaptation, even when the cooling stimulus is still there. On the other hand, for a chemical agent applied to the facial skin, the stimulus is more constantly present. The exact physiological sensation to replicate with the inventive compounds is that of refreshing, soothing coolness, with minimal or no sensations of irritation or sting, and the absence of excessive cold.

[0072] The long durations of action of some preferred NACE compounds on the philtrum skin and the ocular surfaces were unexpected and surprising. Some of the compounds, especially the isopropyl ester derivatives of glycine and D-alanine were found to have cooling actions for three or more hours, an astonishing and unexpected effect.

[0073] Uses of Long-Acting NACE Compounds on the Face and Other Surfaces

[0074] In a preferred use, one or more long-acting NACE compounds is topically applied to therapeutically relieve the irritation, itch, and/or pain of inflamed surfaces. Other contemplated uses include refreshment of the facial skin and to increase alertness and vigilance.

[0075] Therapeutic uses for topical formulations of one or more long-acting NACE compounds are contemplated in a towelette for conjunctivitis, ocular surface irritation, pain from corneal abrasions, and pain from eye surgery.

[0076] Delivery to Target and Utility of N-Alkylcarbonyl-Amino Acid Esters

[0077] In practice, the long-acting NACE compound may also be applied onto the skin using a towelette that is of a construction sufficient or adapted to deliver the NACE compound to the skin. Thus, the desired NACE compound is suspended, dissolved, and/or dispersed so as to be in contact with the towelette. Suitable towelettes include a pad that may be of woven or nonwoven material usually in a unit dispenser. The wiping of the towelette or pad across skin results in delivery to the skin of dermatologically active ingredient(s), meaning that the skin is substantially medicated. Other drugs, cosmeceuticals, herbal medicines, traditional medicines, and active cosmetic ingredients suitable for topical human use may also be incorporated into the towelette.

[0078] The ability of long-acting NACE compound to impart cooling and refreshment in a towelette without sting, burn or irritation (especially to the eyes), is an advance over current technology on cooling agents. Known towelettes frequently contain SD Alcohol (specially denatured alcohol; usually ethanol, isopropyl alcohol or methanol), which is present as a solvent and/or a cooling agent. Alcohol produces cooling when it abstracts heat from its environs during evaporation. The drawback of using short-chain carbon-alcohols in such formulations is that the alcohol dehydrates tissues and causes irritation. When such a towelette is used near the eyeball, the alcohol vapors irritate the eye surface. Similarly, menthol, camphor, eucalyptol, and other ingredients added to towelettes to produce fragrance and cooling also irritate the skin and eyes.

[0079] In one embodiment, a long-acting NACE compound is carried by a towelette, which, for example, when applied to the face, will be especially valuable in counter-acting fatigue and to produce alertness and increased vigilance; for example, to combat tiredness from long car journeys or work in a hot environment.

[0080] Summary of Experimental Results from Bioassays

[0081] The principal findings from experiments performed on the skin are summarized in FIG. **1** and Table 3. The beneficial effects of the long-acting NACE compounds are the long duration of action in the absence of significant eye irritation.

TABLE 2

Summary comparison of unique properties of long-acting NACE compounds with other compounds.

Chemical Class	Cooling on tongue	Cooling on skin of face	"perfect" cooling experience	Eye Irritancy	Acts for >1 hour at 1 mg/ml
Long-acting NACE	yes	yes	yes	no	yes
non-NACE carboxamides	yes	variable	no	yes	no
(−)-menthol	yes	yes	no	yes	no
SD alcohol	no	yes	yes	yes	no

[0082] Chemical Synthesis of N-Alkylcarbonyl-Amino Acid Esters

[0083] Many substituted amino acid esters may be obtained from commercial sources such Sigma-Aldrich Corp., St. Louis, Mo., USA. For example, sarcosine ethyl ester, β-alanine ethyl ester, R- or S-amino butyrolactone, and L- or D-alanine methyl ester, are listed in the 2003-2004 Aldrich Catalog. The precursor, D-alanine ethyl ester is available from Indofine Chemicals, Co., Hillsborough, N.J. The precursor, D-alanine isopropyl ester is not available from commercial sources and was custom synthesized (Phoenix Pharmaceuticals, Burlingame, Calif.). The acid chloride is reacted with the appropriate amino acid ester to form the NACE compound.

[0084] As an example of synthetic procedure, D-Ala methyl ester hydrochloride was obtained from Aldrich Chemical Co., 1.0 g was dissolved in 28 mL diethyether and 1 mL double-distilled water and cooled to 0° C. A pinch of the catalyst diaminopyrimidine was added. 1.62 mL of p-menthoyl chloride was then added dropwise, followed by 2 mL of triethylamine. Clumps of white precipitates appeared in the mixture, which was stirred overnight at room temperature. The precipitate was dissolved with ethyl acetate, washed with double-distilled water, and dried over sodium sulfate. The organic phase was then evaporated under reduced pressure to yield the final product (2 g), which crystallized at room temperature. The expected molecular mass was then confirmed by mass spectroscopy and the absorption spectrum by nuclear magnetic resonance.

[0085] Bioassay Procedures

[0086] For bioassay on the skin, approximately 30 mg was stirred and dissolved in 3 g of warm liquid Aquaphor® ointment to a yield 5 to 10 mg/ml (0.5% to 1.0 wt/vol) ointment. After cooling, 40 to 70 mg of the solid ointment was placed on the tip of a plastic stick and applied to the skin above the upper

lip, on the philtrum, and lateral to the philtrum, up to the nasolabial folds, of test subjects and the onset and duration of cooling sensations noted.

[0087] The intensity of the subjective skin sensation was rated as 0, 1, 2 or 3 with: 0 as no change; 1 as slight coolness, cold, or tingling; 2 as clear signal of coolness, cold, or tingling; and 3 as robust cooling or cold. The intervals for recording sensations were 5 to 10 minutes, until two successive zeroes were obtained. The results (shown in FIG. **1**) are averaged values of 4 to 6 separate trials in the same individual. The data are plotted using SigmaPlot® (Systat Software, Point Richmond, Calif., USA) and a smoothing function with a negative exponential was used for analysis and statistical fit of the results.

[0088] The onset of drug action was taken as the time to reach 2 units of coolness intensity, and offset of drug action was the time when coolness intensity drops below 2, after previously surpassing 2 units. The duration of cooling action was defined as the offset time minus the onset time. An inactive compound is defined as one that did not exceed 2 units of cooling after application. The quality of the sensation was also noted: such as pure refreshing coolness, or if the sensation was accompanied by irritation (stinging or burning). The quality of the sensation was not rated for intensity.

[0089] For tests of irritancy near the eyes in Study 3 and 4, the ointment was applied to the periorbital skin (upper and lower eyelids and on skin adjacent to the lateral canthus), and the subject asked if irritation is present or absent. The intensity of the eye sensation is not rated, but just noted as being present or absent

[0090] For testing of cooling compounds delivered to the eyelids with a towelette, the following procedures were used in the experiments of Table 3 and in Study 5 and 6. Three ml of liquid suspension or solution in distilled water was added to a 0.4 g cotton rectangle (50 mm×60 mm) (CS-being, Daisan Cotton, Japan) and individually sealed with a vaccuum apparatus (Foodsaver®, Jarden Corp.). Samples were stored in the freezer or refrigerator and thawed to room temperature before use. Wiping with the cotton pad delivers 40 to 45 μl of liquid composition to the eyelids (~20 to 22 μl per eye). So, if the concentration of the test substance was 1 mg/ml, the dose delivered to both eyes is a total of ~40 to 42 μg, or ~20 μg per eye. This method allows a reliable delivery of test substances because the dissolved or suspended particles in solution are evenly dispersed on the cotton pad in an excess volume of liquid. The presence or absence of cooling sensations was noted as being present or absent at 5 to 10 min intervals until no coolness was noticeable in two successive test intervals. Only the duration of cooling on the ocular surface was recorded, without an attempt to quantify the intensity of the sensation.

[0091] Study 1

[0092] A number of compounds were synthesized and tested with the results are shown in FIG. **1**. For the results in FIG. **1**, the test compounds were singly applied to the skin above the upper lips at a 40 to 70 mg dose of a 1% wt/vol (10 mg/ml) ointment. Subsequently, for the results shown in Table 3, the test dose on the philtrum was reduced to a 0.5% wt/vol (5 mg/ml) ointment. The reason for choosing a lower dose was to increase the number of trials per individual and to have a reduced chance of substances accumulating in the skin.

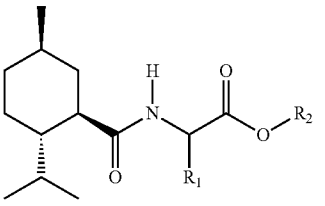
[0093] In FIG. **1**, the duration of cooling effects of some known agents, e.g., (-menthol, WS-3 and WS-5 are 0.3, 0.3, and 0.5 hour, respectively, is relatively short compared to the NACE compounds, specifically, D-Hsl, D-Ala-OMe and D-Ala-OEt analogs with 1.3, 1.9, and 2.4 hours of cooling, respectively.

[0094] Long-acting NACE derivatives that have a refreshing cool, without skin or eye irritancy (a "perfect cool") after facial skin or periorbital applications, are identified by (*) in Table 3 and Table 4. The exceptional long-acting properties of the isopropyl analogs (see Table 1 and Table 3) are noted and not predictable from the known physico-chemical properties of these molecules (Table 4).

TABLE 3

Test results of substances applied to the philtrum skin in an ointment vehicle and to the eylids with a towelette. The test concentrations for the philtrum was 5 mg/ml in Aquaphor ® ointment and the concentrations for the eye wipes was 1 mg/ml in 5%-95% v/v ethanol-distilled water. The duration of cooling is recorded as (minutes).

Formula (1)



Compounds	R_1	R_2	Philtrum Skin (minutes)	Eyelids/Ocular Surface (minutes)
Gly Et Ester (WS-5)	H	Et	24	15
Gly iPr Ester (*)	H	iPr	27	300
Gly nPr Ester	H	nPr	42	54
Gly nBu Ester	H	nBu	38	35
D-Ala Me Ester (*)	Me	Me	77	120
D-Ala Et Ester (*)	Me	Et	103	180
D-Ala iPr Ester (*)	Me	iPr	34	360
D-Ala nPr Ester	Me	nPr	108	65

TABLE 3-continued

D-Ala nBu Ester	Me	nBu	80	40
L-Ala Et Ester	Me	Et	34	0
D-NMe Ala Ester	Me	Me	0	0

(*) denotes compounds that fulfill the criteria of being a long-acting NACE compound for towelette applications (i.e., >1 hour duration of action).

TABLE 4

Some properties of tested compounds. The tongue threshold values are from Watson US 04178459. The pure (*) compounds were never synthesized by Watson et al.

Compound	Molecular Weight	Threshold tongue (μg)	Log P
Gly Me Ester (WS-31)	255.4	0.6	2.4
Gly Et Ester (WS-5)	269.4	0.2	2.9
Gly n-Pr Ester	283.4	0.3	3.4
Gly i-Pr Ester (*)	283.4	—	3.3
Gly n-Bu Ester	297.4	—	4.0
Sar Et Ester	283.4	0.8	2.9
L-Ala Et Ester	283.4	0.4	3.1
L-Ala Me Ester	269.4	0.6	2.8
D-Ala Me Ester (*)	269.4	—	2.8
D-Ala Et Ester (*)	283.4	—	3.1
D-Ala i-Pr Ester (*)	297.4	—	3.7
D-Ala n-Pr Ester	297.4	—	3.8
D-Ala n-Bu Ester	311.4	—	4.4
N—Me-D-Ala Et Ester	297.4	—	3.2
β-Ala Et Ester	283.4	1.5	3.1
D-Hsl	267.4	—	2.5
L-Hsl	267.4	—	2.5
racemic Hsl	267.4	—	2.5
WS-3	211.3	0.2	3.7
WS-10	225.4	0.4	4.1
WS-34	239.4	0.7	4.6
WS-14	239.4	0.4	4.5
WS-11	255.4	0.3	2.9
WS-12	289.4	0.2	5.3
L-Ser Et Ester	285.4	—	1.8
L-Val Me Ester	297.4	—	4.2
D-Val Me Ester	297.4	—	4.2
Glu(OMe) Me Ester	341.4	—	2.2
L-Leu Me Ester	311.5	—	4.3
L-Pro Me Ester	295.4	—	3.3
L-Lys(Z) t-Bu Ester	502.7	—	5.9
L-Tyr Me Ester	364.2	—	3.7

(*) denotes compounds that fulfill the criteria of being a long-acting NACE compound for towelette applications (i.e., >1 hour duration of action). (Gly = glycine; Sar = sarcosine; Ala = alanine; Hsl = homoserine lactone (also known as α-amino-butyro-γ-lactone))

[0095] Study 2

[0096] 2-Isopropyl-5-methyl-cyclohexanecarbonyl D-alanine methyl ester was dissolved in warm 10% propyleneglycoV90% distilled water solution to give a concentration of either 0.1 or 0.5% wt/vol (1 or 5 mg/mL). Six to seven milliliters of these solutions were then applied with a pipette onto a paper napkin (Luncheon Napkins, Kirkland Signature brand from Costco, Inc.) The napkin was 1-ply with a 30.4× 29.5 cm dimension. Each napkin was then hermetically sealed in a plastic envelope (Foodsaver by Tilia). On two separate occasions involving golfing trips to Los Angeles, Calif., USA, where air temperatures exceeded 332° C. (90° F.), these towelettes were used to wipe the face. Pleasant cooling sensations were obtained that lasted for about 10 minute for the 0.1% concentration towelette and about 60 minutes for the 0.5% towelette. No eye irritation was observed with either concentration.

[0097] Study 3

[0098] A 1% preparation of 2-isopropyl-5-methyl-cyclohexanecarbonyl D-alanine methyl ester in Aquaphor® ointment was applied bilaterally to the periorbital area of four individuals. Onset of coolness on the skin was noted with 1 minutes and lasting for an average of 30 minutes. No irritation of the eye surfaces was noted. Surprisingly, all four individuals noted an alerting effect and an ability to focus and see more clearly. This alerting effect lasted for about one hour. This experiment was then repeated three days later in the evening with the same individuals whose occupations required daylong activities before a computer screen. Again, application of the ointment about the periorbital area was reported to relieve fatigue, enhance visual acuity, and to increase attention span and focus. Sensations of refreshment and improved mood were also noted. By contrast, tests with other carboxamides, WS-3, WS-23, WS-11, and WS-14, under similar conditions, showed that they produced significant skin and eye irritation and did not enhance skin coolness or provide satisfactory refreshment

[0099] Study 4

[0100] Three high-powered business executives who regularly spent long hours at the negotiating tables or at meetings felt "burnt out" by their professional activities and frequently felt tired and sleepy-eyed in the social arena. They volunteered to try eye wipes containing 1.0 mg/ml of (R)-2-[((1R, 2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid isopropyl ester (D-Ala-OiPr, No. 1, in table 1) to see if the eye wipes will refresh and awaken their interest during meetings. All three said when coffee was no longer able to keep them awake, they went to the bathroom and applied the eye wipe and the sense of sleepiness and fatigue was reduced for the next several hours. They became alert and vigilant and more pro-active in their social milieu. Similar results were obtained with the Gly-OiPr, D-Ala-OMe, and D-Ala-OEt analogs. It was generally opined that the latter two analogs had a faster onset and more intense awakening effect.

[0101] Study 5

[0102] A laboratory scientist suffered from seasonal allergic conjunctivitis. This condition was severely aggravated when he started doing experiments with laboratory animals (rats and mice) and he became sensitized to the associated allergens. The conjunctivitis was not relieved by oral or topical eye drop antihistamines and the subject was reluctant to consider the use of anti-inflammatory steroid ointments. Upon examination, his eyes were blood-shot and watery and he kept rubbing his eyelids with his fingers even though he knew this action may further aggravate the itch and discomfort. He volunteered to try eye wipes containing 1.0 mg/ml of (R)-2-[((1 R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-acetic isopropyl ester (Gly-OiPr, No. 2, in table 1). The relief of itch and discomfort was obtained within 5 minutes and lasted for at least four hours. He used the wipes

on an "as-needed basis" for three days and was surprised to see that his over-all itchiness and redness were reduced significantly.

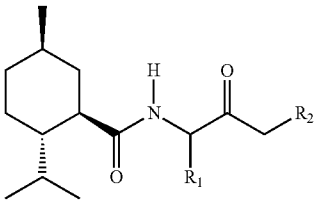
[0103] Study 6

[0104] A 60-year old woman suffered from "dry eyes" or keratoconjunctivitis sicca which was managed by artificial tears and eye drops containing lubricants. She then underwent cataract surgery and started to complain of persistent severe periorbital pain. She will wake up at night in severe discomfort and not be able to sleep. This condition persisted for six months and her quality of life deteriorated and she began to lose weight. Consultation with the ophthalmologist who performed the surgery was not helpful, as he could not detect any physical signs of injury to the eye. Pain in the "quiet eye" (see Brazis et al. Clinical review: the differential diagnosis of pain the quiet eye. The Neurologist 8: 82-100, 2002) is a well-known syndrome with many causes. She volunteered to try eye wipes containing 1.0 mg/ml of (R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-acetic isopropyl ester (Gly-OiPr, No. 2, in table 1) and she obtained immediate relief on three occasions and was able to go to sleep. Subsequently, she went to another ophthalmogist who specialized in corneal diseases. He diagnosed her condition as infective blepharitis and prescribed a topical antibiotic and a topical anti-inflammatory steroid. These medications have now restored her health.

[0105] The foregoing has described the principles, preferred embodiments, and modes of operation of the present invention. However, the invention should not be construed as limited to the particular embodiments discussed. Instead, the above-described embodiments should be regarded as illustrative rather than restrictive, and it should be appreciated that variations may be made in those embodiments by workers skilled in the art without departing from the scope of the present invention.

1. A towelette containing a compound of Formula (1):

Formula (1)



wherein:

R_1 is hydrogen, or a methyl group such that the carbon atom to which it is attached is in the D-configuration, and

when R_1 is hydrogen, R_2 is isopropyl, and

when R_1 is a methyl group such that the carbon atom to which it is attached is in the D-configuration, R_2 is a C_1 to C_3 alkyl group.

2. A compound according to claim **1**, which is (R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid methyl ester.

3. A compound according to claim **1**, which is (R)-2-R(1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid ethyl ester.

4. A compound according to claim **1**, which is (R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-propionic acid isopropyl ester.

5. A compound according to claim **1**, which is (R)-2-[((1R,2S,5R)-2-isopropyl-5-methyl-cyclohexanecarbonyl)-amino]-acetic acid isopropyl ester.

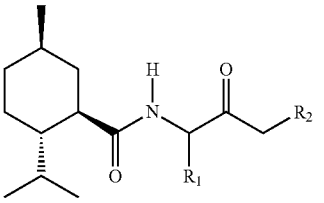
6. The towelette as in claim **1** wherein the compound is present in the towelette at a liquid concentration of 0.2 to 2 mg/ml.

7. A method of preparing a therapeutic article of manufacture, comprising:

providing a towelette;

contacting the towelette with a compound of Formula (1):

Formula (1)



wherein:

R_1 is hydrogen, or a methyl group such that the carbon atom to which it is attached is in the D-configuration, and

when R_1 is hydrogen, R_2 is isopropyl, and

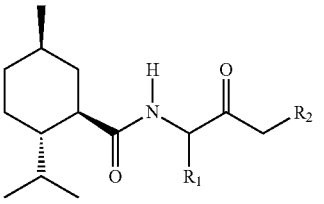
when R_1 is a methyl group such that the carbon atom to which it is attached is in the D-configuration, R_2 is a C_1 to C_3 alkyl group.

8. Use of towelette according to claim **7** to alleviate ocular skin irritation, itch, and/or pain, and/or to increase alertness and to reduce sleepiness and fatigue.

9. A therapeutic method comprising:

contacting a towelette carrying a compound of Formula (1):

Formula (1)



wherein:

R_1 is hydrogen, or a methyl group such that the carbon atom to which it is attached is in the D-configuration, and

when R_1 is hydrogen, R_2 is isopropyl, and

when R_1 is a methyl group such that the carbon atom to which it is attached is in the D-configuration, R_2 is a C_1 to C_3 alkyl group.

with the facial skin of a mammal, the contacting sufficient to deliver a therapeutically effective amount of the formula 1 compound.

10. The method as in claim **9** wherein the therapeutically effective amount is sufficient to alleviate ocular skin irritation, itch, and/or pain, and/or to increase alertness and to reduce sleepiness and fatigue.

* * * * *