Drugs of the Future 2004, 29(5): 441-448

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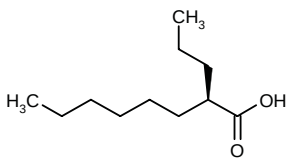
Arundic Acid

Rec INN

Astrocyte-Modulating Agent
Treatment of Stroke
Treatment of Neurodegeneration

ONO-2506
ONO-2506PO
Cereact®
Proglia®

2(*R*)-Propyloctanoic acid



$C_{11}H_{22}O_2$
Mol wt: 186,2928
CAS: 185517-21-9
EN: 258543

Abstract

According to the World Health Organization, stroke is the leading cause of death worldwide, accounting for 5 million deaths per year. Oxygen deprivation due to stroke leads to rapid nerve cell death and dysfunction of the body part controlled by the affected nerve cells. Thus, stroke is also responsible for serious long-term disability (*e.g.*, paralysis, cognitive deficits, dementia, dizziness, vertigo, impaired vision, language deficits, emotional difficulties, pain). Although there have been improvements in recent years in the treatment of stroke, the need for novel therapies to prevent and treat stroke remains a research priority. One novel agent to emerge is Ono-2506 (arundic acid), which modulates astrocyte activation by inhibiting the enhanced astrocytic synthesis of S-100β, responsible for inducing neuronal death. Ono-2506 does not affect thrombi or blood vessels and therefore does not pose a risk for hemorrhage. It has shown efficacy in preventing expansion of cerebral infarction by improving astrocyte function and may be effective even when administered hours after ischemic stroke onset. Ono-2506 is undergoing phase II development for the treatment of acute ischemic stroke, as well as clinical development in other neurodegenerative diseases including amytrophic lateral sclerosis, Alzheimer's disease and Parkinson's disease.

Synthesis

Arundic acid can be prepared by several ways:

1) Alkylation of dimethyl malonate (I) with hexyl bromide (II) by means of NaOMe in methanol gives dimethyl 2-hexylmalonate (III), which is alkylated again with 2-propynyl bromide (IV) by means of NaOMe in methanol to yield dimethyl 2-hexyl-2-(2-propynyl)malonate (V). Selective monodecarboxylation of malonate (V) by means of LiCl in hot DMSO affords methyl 2-(2-propynyl)octanoate (VI), which is hydrolyzed to the corresponding carboxylic acid (VII) by means of NaOH in methanol. Then, racemic acid (VII) is submitted to optical resolution by crystallization with 1(*R*)-phenylethylamine (VIII), followed by hydrolysis with HCl to provide 2(*S*)-(2-propynyl)octanoic acid (IX), which is finally hydrogenated with H_2 over Pd/C in ethyl acetate (1). Scheme 1.

2) Alternatively, the racemic acid (VII) can also be obtained by alkylation of ethyl octanoate (X) with 2-propynyl bromide (IV) by means of LDA in HMPA/THF to give ethyl 2-(2-propynyl)octanoate (XI) and then hydrolysis with NaOH in ethanol (1). Scheme 1.

3) Reaction of L-prolinol (XII) with pentanoyl chloride (XIII) by means of triethylamine (TEA) in dichloromethane gives *N*-pentanoyl-L-prolinol (XIV), which is stereoselectively alkylated with hexyl iodide (XV) by means of butyl lithium and diethylamine (DEA) in THF to yield *N*-[2(*R*)-propyloctanoyl]-L-prolinol (XVI). This compound is subjected to purification via its esterification with 4-nitrobenzoyl chloride (XVII) and pyridine to the corresponding ester (XVIII) and recrystallization to form *n*-hexane. Hydrolysis of the pure ester (XVIII) with LiOH in water affords pure *N*-[2(*R*)-propyloctanoyl]-L-prolinol (XVI), which is finally treated with HCl in AcOH (2). Scheme 2.

4) Acylation of (–)-2,10-camphorsultam (XIX) with octanoyl chloride (XX) by means of TEA and DMAP in THF provides the *N*-octanoylcamphorsultam (XXI), which

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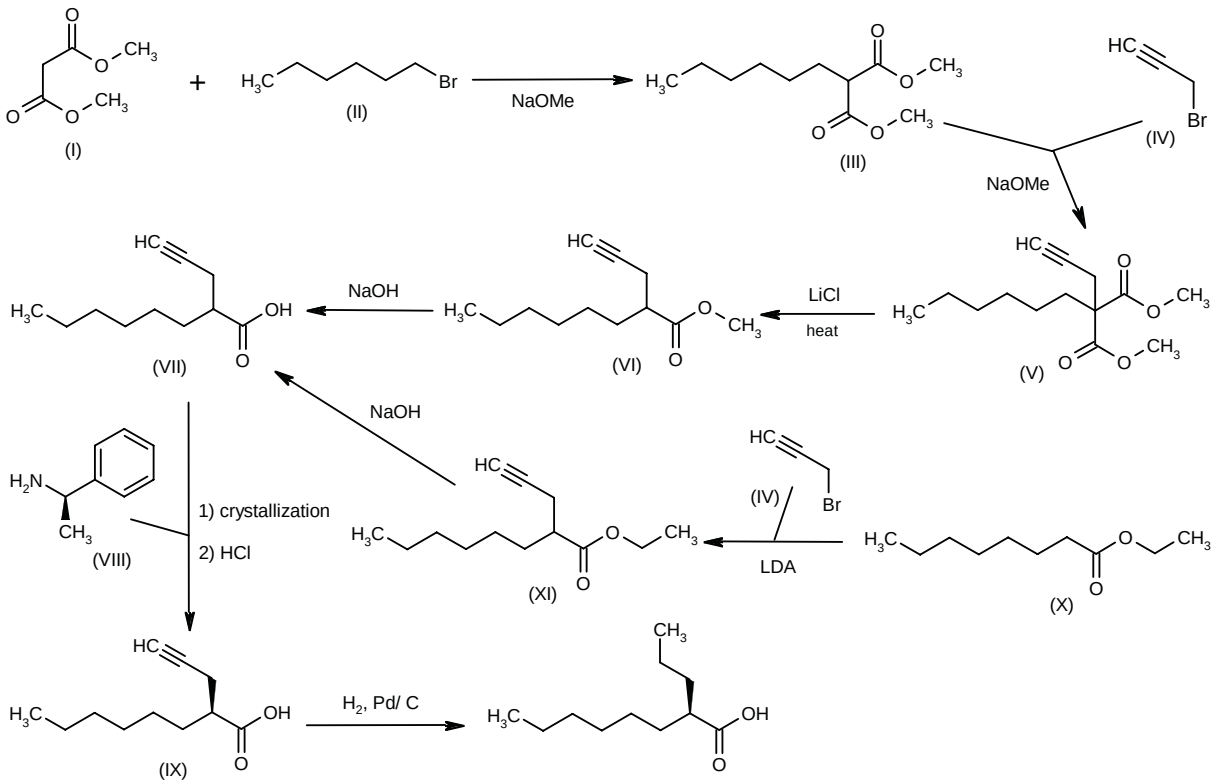
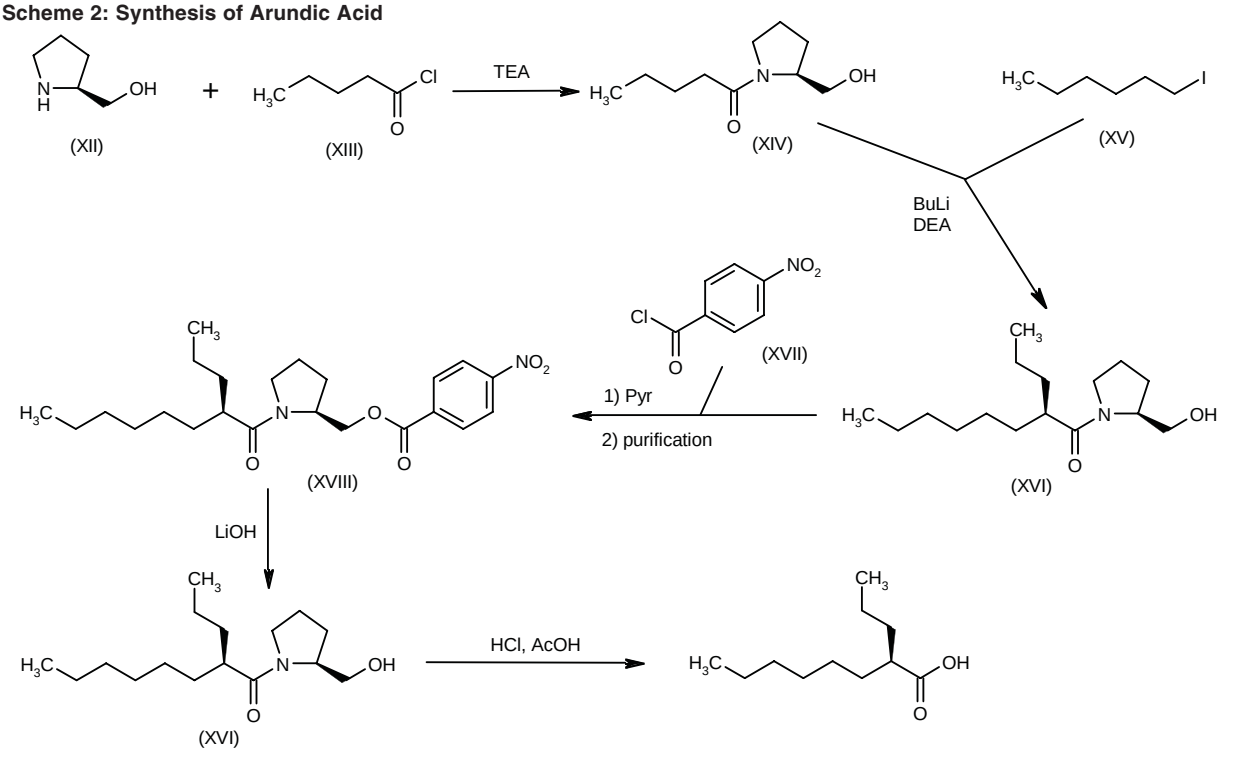
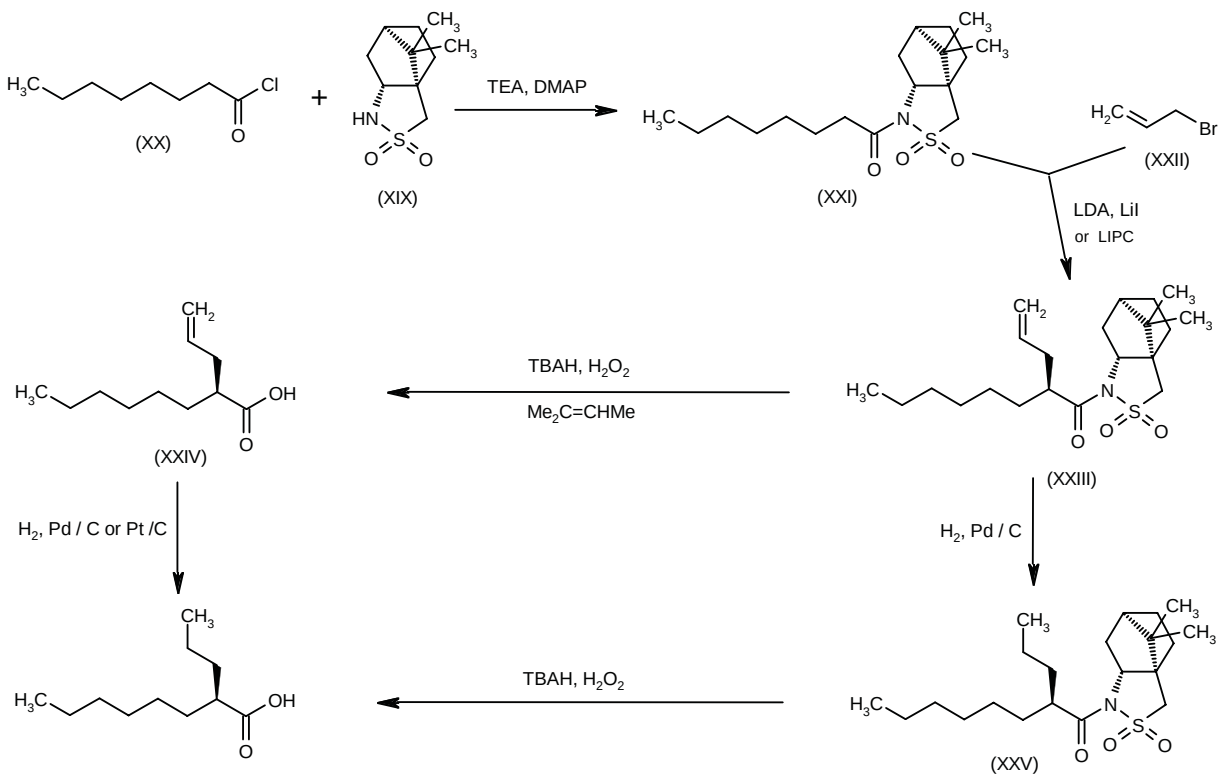
Scheme 1: Synthesis of Arundic Acid



Scheme 2: Synthesis of Arundic Acid



Scheme 3: Synthesis of Arundic Acid



is diastereoselectively alkylated with allyl bromide (XXII) by means of LDA (prepared *in situ* from diisopropylamine and BuLi) and LiI (3-5) or LIPC (prepared *in situ* from isopropylcyclohexylamine and BuLi) (5) in THF to give the *N*-[2(*S*)-(2-propenyl)octanoyl]camphorsultam (XXIII). Hydrolysis of this *N*-acylsultam (XXIII) with tetrabutylammonium hydroxide (TBAH), H_2O_2 and 2-methyl-2-butene in DME gives the carboxylic acid (XXIV) (3, 4), which can be purified by crystallization with cyclohexylamine in AcOEt in order to improve the final product quality (5, 6). Finally, carboxylic acid (XXIV) is submitted to catalytic hydrogenation in the presence of either Pt/C in *i*-PrOH (3, 5, 6) or Pd/C in AcOEt/MeOH (4). Scheme 3.

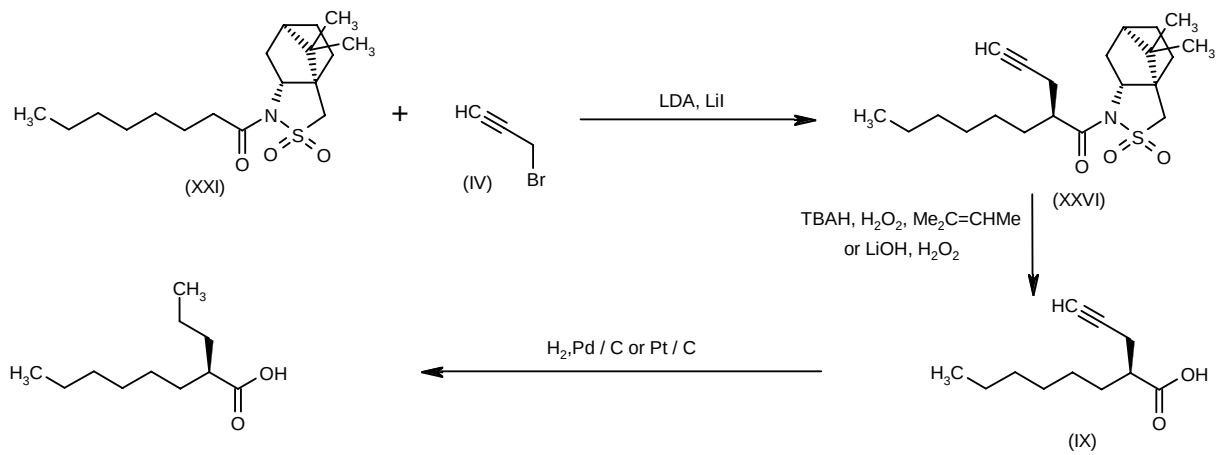
5) Alternatively, hydrogenation of the *N*-acylsultam (XXIII) under Pd/C in AcOEt/MeOH provides the saturated derivative (XXV), which is finally submitted to camphorsultam hydrolytic removal by means of TBAH and H_2O_2 in THF (3, 4). Scheme 3.

6) In a related procedure, the *N*-octanoylcamphorsultam (XXI) is alkylated with propargyl bromide (IV) and LDA and LiI in THF to produce compound (XXVI), which is hydrolyzed by means of H_2O_2, TBAH and 2-methyl-2-butene in DME (3, 4) or LiOH and H_2O_2 in THF/H_2O (4) to furnish the 2-propargyloctanoic acid (IX). Finally, this compound is catalytically hydrogenated in the presence of either Pt/C in *i*-PrOH (3, 6) or Pd/C in AcOEt or diethoxyethane (4). Scheme 4.

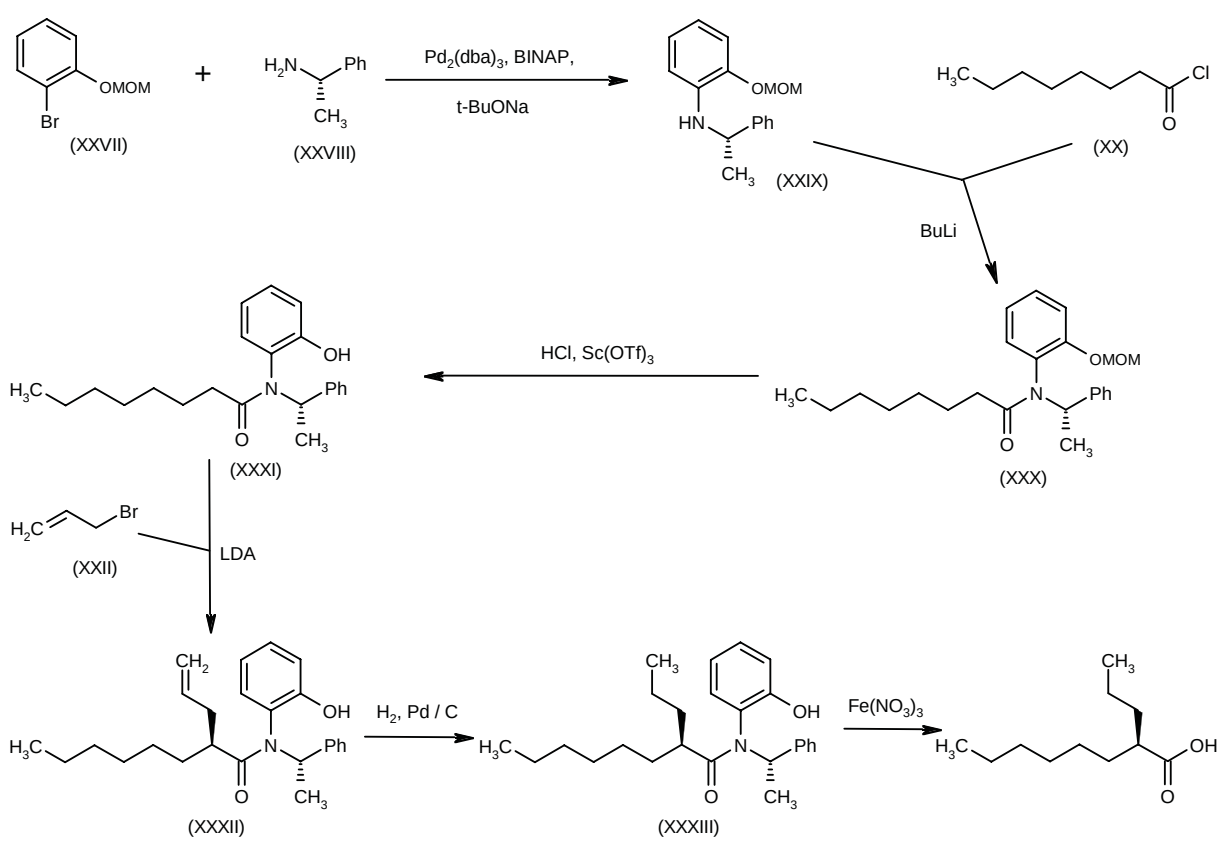
7) Reaction of 1-bromo-2-(methoxymethoxy)benzene (XXVII) with 1(*S*)-phenylethylamine (XXVIII) by means of $Pd_2(dba)_3$, BINAP and *t*-BuONa in toluene gives the chiral secondary amine (XXIX), which is condensed with octanoyl chloride (XX) by means of BuLi in THF to yield the octanamide (XXX). Cleavage of the MOM protecting group of compound (XXX) by means of HCl and $Sc(OTf)_3$ in methanol affords *N*-(2-hydroxyphenyl)-*N*-[1(*S*)-phenylethyl]octanamide (XXXI), which is stereoselectivelly alkylated with allyl bromide (XXII) and LDA in THF to provide the chiral 2-allyloctanamide (XXXII). Hydrogenation of amide (XXXII) with H_2 over Pd/C in methanol yields the corresponding 2-propyl derivative (XXXIII), which is finally submitted to the cleavage of the chiral auxiliary by means of $Fe(NO_3)_3$ in refluxing dioxane/water (7). Scheme 5.

8) Acylation of 4(*R*)-benzyloxazolidin-2-one (XXXIV) with octanoyl chloride (XX) by means of *t*-BuOK in THF gives 3-octanoyl-4(*R*)-benzyloxazolidin-2-one (XXXV), which is alkylated with allyl iodide (XXXVI) and LiHMDS in THF to yield the chiral 2-allyloctanoyl amide (XXXVII). Hydrogenation of amide (XXXVII) with H_2 over Pd/C in ethanol affords the corresponding 2-propyl derivative (XXXVIII), which is finally submitted to hydrolysis of the chiral auxiliary (8). Scheme 6.

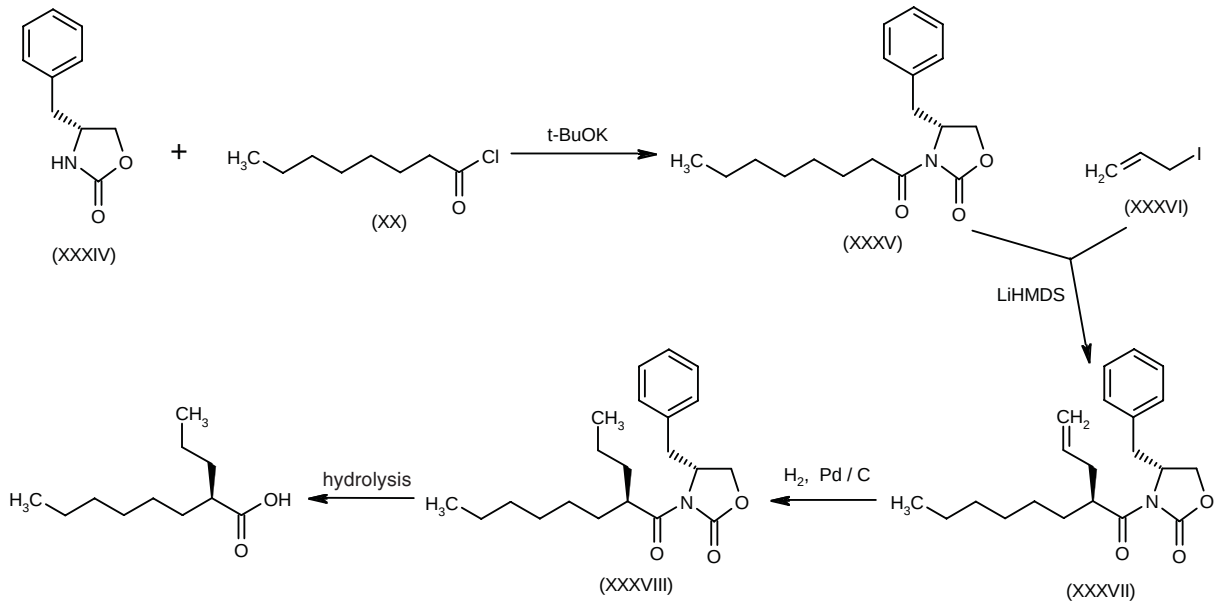
Scheme 4: Synthesis of Arundic Acid



Scheme 5: Synthesis of Arundic Acid



Scheme 6: Synthesis of Arundic Acid



Introduction

Stroke is the abrupt impairment of brain function resulting from the occlusion or rupture (from a thrombus, atherosclerotic plaque or other particle) of intra- or extracranial blood vessels. Because cerebral nerve cells cannot store oxygen and require a constant supply to function, oxygen deprivation due to stroke leads to rapid nerve cell death and dysfunction of the body part controlled by the affected nerve cells. Nerve cells cannot regenerate and are replaced by a fluid-filled cavity or infarct. Stroke causes the immediate death of some nerve cells, while other cells in the penumbra are acutely damaged and remain in a compromised state for a number of hours. According to the American Heart Association, 700,000 Americans suffer a stroke every year and the incidence is rising, probably due to the increasing elderly population. About 500,000 cases of diagnosed stroke are first attacks and the other 200,000 are recurrent strokes. Similar numbers occur in Europe and the World Health Organization (WHO) has identified stroke as the leading cause of death worldwide, accounting for 5 million deaths per year (9, 10).

There are 4 major types of stroke. Cerebral thrombosis and cerebral embolism are ischemic strokes caused by blockage of blood vessels and are responsible for almost 80% of all cases. Subarachnoid hemorrhage and intracerebral hemorrhage are hemorrhagic strokes usually caused by bleeding or hemorrhage due to aneurysm or head injury. Other types of stroke include transient ischemic attacks (TIAs) or ministrokes, which begin as full-blown strokes but resolve quickly and leave no noticeable symptoms or deficits, and lacunar infarcts, which are a series of extremely small ischemic strokes that lead to clumsiness, weakness and emotional lability (9).

Stroke not only is a leading cause of death, but also is responsible for serious long-term disability in surviving individuals. Disabilities due to neuronal death and injury can include paralysis of one or both sides of the body, cognitive deficits, dizziness, vertigo, impaired vision, language deficits, emotional difficulties and pain. A high risk of dementia has also been noted in patients with ischemic stroke (9, 11, 12).

Although there have been improvements in recent years in the treatment of stroke, prevention is the best approach for limiting stroke mortality and morbidity. The development of newer therapies and effective risk reduction have shown considerable promise. However, the need for novel therapies to prevent and treat stroke remains a research priority (13, 14).

One novel agent to emerge that shows particular promise is Ono-2506 (arundic acid), the enantiomerically pure (*R*)-2-propyloctanoic acid. Ono-2506 was discovered following screening of compounds for their ability to inhibit astrocytic synthesis of S-100β. Enhanced S-100β synthesis by periinfarct-reactive astrocytes has been observed, inducing neuronal death via nitric oxide (NO) release. Thus, S-100β is suspected of playing an important role in the occurrence of delayed infarct expansion (15-18). Ono-2506 modulates astrocyte activation but does not affect thrombi or blood vessels and therefore

does not pose a risk for hemorrhage. It was chosen for further development to prevent expansion of cerebral infarction by improving astrocyte function, with possible efficacy even when administered hours after ischemic stroke onset.

Pharmacological Actions

Ono-2506 was shown to have numerous effects on astrocyte function *in vitro*. Studies using cultured astrocytes prepared from neonatal rat cerebral cortex reported that treatment with the agent concentration-dependently inhibited S-100β content and nerve growth factor (NGF) secretion, and facilitated the expression of glutamate transporters (GLT-1 and GLAST) and the $GABA_A$ receptor. The compound significantly increased total glutathione (GSH) content, protected cells from death and increased the oxidized GSH:total GSH ratio induced by hydrogen peroxide. Pretreatment with Ono-2506 also inhibited lipopolysaccharide (LPS)-induced increases in inducible NO synthase (iNOS) and cyclooxygenase type 2 (COX-2) mRNA. Ono-2506 therefore appears to modulate astrocyte activation and function (19-23).

Ono-2506 was also shown to exert neuroprotective effects *in vivo* in animal models of ischemic stroke, Parkinson's disease and Alzheimer's disease. A study in rats subjected to permanent middle cerebral artery occlusion (MCAO) demonstrated that treatment with Ono-2506 (10 mg/kg/day i.v. starting immediately after MCAO and continuing for 7 days) did not affect acute infarct expansion (up to 24 h post-MCAO) but abolished delayed infarct expansion between 24 and 168 h post-MCAO. Treatment significantly decreased the expression of S-100β and glial fibrillary acidic acid protein (GFAP) in activated astrocytes and reduced the number of TUNEL-positive cells in the periinfarct area. Moreover, neurological deficits, as determined by performance in the rotarod test, were significantly improved as soon as 24 h after the first Ono-2506 dose. The therapeutic window for the agent was 0-48 h after MCAO (24).

The severe neurological deficits in rats subjected to thrombotic focal cerebral ischemia were also significantly improved with administration of a combination of Ono-2506 (10 mg/kg by i.v. bolus daily starting 40 min postthrombosis) and recombinant tissue plasminogen activator (rtPA; 100,000 IU infused over 15 min starting 10 min after thrombosis) (25).

Results from a study using freely moving rats subjected to microdialysis of the right frontal cortex and 1-h MCAO showed that i.v. treatment with Ono-2506 6 h post-MCAO attenuated the delayed increase in glutamate levels, possibly via inhibition of the reduction in GLT-1 and GLAST expression. Ono-2506 may therefore prevent infarct formation following ischemia via inhibition of extracellular glutamate increases (26).

The efficacy of Ono-2506 (3 and 10 mg/kg/day i.v. starting 6 h post-MCAO and continuing for 6 days) in improving neurological symptoms following focal ischemia was demonstrated in cynomolgus monkeys subjected to permanent MCAO. Treatment with the agent not only reduced focal and diffused neuronal necrosis and degeneration in the outer boundary zone of the infarct area, but also ameliorated neurological symptoms (27).

Ono-2506 was also effective in a mouse model of Parkinson's disease. Neuroprotective effects of Ono-2506 were observed against MPTP-induced neurotoxicity in C57B1/6 mice. Treatment with Ono-2506 (30 mg/kg i.p. at 1, 6, 24 and 48 h after MPTP injection) significantly increased striatal dopamine content (51%) as compared to MPTP-treated controls; pretreatment or a single dose of Ono-2506 at 6 h post-MPTP injection was ineffective. Animals treated with Ono-2506 did not exhibit the MPTP-induced reduction in tyrosine hydroxylase-positive dopaminergic neurons in the substantia nigra at 3 days. In addition, treatment induced earlier astroglial activation as compared to untreated controls. Thus, Ono-2506 protects dopaminergic neurons from MPTP neurotoxicity by improving astroglial function (28).

Overexpression of S-100β has also been observed in astrocytes in senile plaques in Alzheimer's disease, suggesting the possible efficacy of Ono-2506 in this indication. An *in vitro* study using human glioblastoma astrocytoma U-373 MG cells showed that treatment with Ono-2506 significantly inhibited β-amyloid(1-40) and β-amyloid(1-42) production, as well as amyloid precursor protein (APP) expression. The agent had no effect on α-, β- or γ-secretase activity. It was concluded that Ono-2506 inhibited β-amyloid production via restoration of astrocytic activation by suppressing APP and S-100β overexpression (29).

A study using senescence-accelerated mice (SAMP8; 12-14 months old), an animal model of neurodegenerative diseases manifesting as dementia, showed that treatment with Ono-2506 improved cognitive dysfunction. Although acute (1 h before testing) or chronic (for 2 months followed by a 1- or 2-week washout period before testing) treatment with Ono-2506 or donepezil had no significant effects on spatial memory impairment in the water maze task, chronic Ono-2506 exerted a beneficial effect. Treatment with Ono-2506 or donepezil for 1 week prior to testing improved cognitive deficits in a passive avoidance task. However, whereas once-daily treatment with Ono-2506 for 2 months followed by a 2-week withdrawal period significantly improved memory failure, donepezil had no effect in the passive avoidance paradigm. Thus, the beneficial effects on learning impairment seen with Ono-2506 and donepezil appear to be via different mechanisms of action (30).

Clinical Studies

A prospective, randomized, blinded, placebo-controlled phase I trial in 92 patients with ischemic stroke examined the safety, tolerability and efficacy of Ono-2506

(2, 4, 6, 8, 10 or 12 mg/kg/h daily starting within 24 h of cerebral infarction and continuing for 7 days). The study included a follow-up period ending at 40 days. Analysis of serum samples from 86 patients revealed that peak S-100β production on day 3 after cerebral infarction was suppressed by Ono-2506 treatment; significant and maximum differences in S-100β levels between Ono-2506- and placebo-treated patients were observed at 7 and 12 h postinfusion on day 3. A strong correlation was observed between baseline NIH Stroke Scale (NIHSS) scores and maximal S-100β levels in both groups. The mean NIHSS tended to be lower in patients treated with Ono-2506 as compared to placebo (12.9 ± 4.6 *vs.* 14.3 ± 5.1). A significant reduction in mean NIHSS was observed in the 8 mg/kg Ono-2506 group as compared to placebo on days 3, 7, 10 and 40. Significantly more patients receiving 8 mg/kg Ono-2506 achieved total functional recovery as compared to placebo (37.5% *vs.* 2.5%). In addition, significantly more patients had favorable outcomes in the 8 mg/kg Ono-2506 group as compared to placebo on days 10 and 40. It was concluded that Ono-2506 was well tolerated and associated with less mortality as compared to placebo (2 *vs.* 5 patients died by day 40). Adverse events were similar in both treatment and placebo groups, with headache the most commonly reported event (32.7% and 39.5% in Ono-2506 and placebo groups, respectively). Four and 5 patients in the Ono-2506 and placebo groups, respectively, discontinued early for adverse events. It was concluded that Ono-2506, through postischemic modulation of astrocyte activation, suppresses S-100β, which may be a marker of lesion volume in ischemic brain injury (31, 32).

Ono-2506 is being evaluated in phase II clinical trials in Japan and North America as an injectable formulation (Proglia®) for the treatment of acute ischemic stroke. An oral formulation (Cereact®) is also in phase II trials in Europe for amyotrophic lateral sclerosis, and phase II studies are being prepared in Japan in patients with Parkinson's disease and in the U.S. in patients with Alzheimer's disease (33).

Source

Ono Pharmaceutical Co., Ltd. (JP).

References

1. Nakai, H., Kishimoto, K. (Ono Pharmaceutical Co., Ltd.). *Preparation method of optically active 2-propyl octanoate.* JP 1996291106.

2. Nakai, H., Kishimoto, K. (Ono Pharmaceutical Co., Ltd.). *Preparation method of optically active 2-propyloctanoic acid.* JP 1996295648.

3. Hasegawa, T., Yamamoto, H. *A practical synthesis of optically active (R)-2-propyloctanoic acid: Therapeutic agent for Alzheimer's disease.* Bull Chem Soc Jpn 2000, 73: 423-8.

4. Yamamoto, H. (Ono Pharmaceutical Co., Ltd.). *Novel intermediates and processes for the preparation of optically active octanoic acid derivs.* EP 1078921, US 6333415, WO 9958513.

5. Hasegawa, T., Kawanaka, Y., Kasamatsu, E., Iguchi, Y., Yonekawa, Y., Okamoto, M., Ohta, C., Hashimoto, S., Ohuchida, S. *Process development of ONO-2506: A therapeutic agent for stroke and Alzheimer's disease.* Org Process Res Dev 2003, 7: 168-71.

6. Toda, N., Yamaguchi, K., Iguchi, Y. (Ono Pharmaceutical Co., Ltd.; Tokyo Kasei Kogyo Co., Ltd.). *Process for the preparation of (2R)-2-propyloctanoic acid.* EP 1153910, WO 0048982.

7. Hasegawa, T., Yamamoto, H. *Development of new chiral auxillary derived from (S)-(–)-phenylethylamine for a synthesis of enantiopure (R)-2-propyloctanoic acid.* Synthesis (Stuttgart) 2003, (8): 1181-6.

8. Tatsuta, K. (Ono Pharmaceutical Co., Ltd.). *Intermediate and process for producing optically active cpd. from the intermediate.* WO 0351852.

9. Prous Science Drug R&D Backgrounders: *Stroke (online publication).* Updated May 25, 2004.

10. Alberts, M.J. *Treatment of acute ischemic stroke.* J Stroke Cerebrovasc Dis 2001, 10(2, Part 2): 10-7.

11. Bravata, D.M., Ho, S.-Y., Brass, L.M., Concato, J., Scinto, J., Meehan, T.P. *Long-term mortality in cerebrovascular disease.* Stroke 2003, 34: 699-704.

12. Desmond, D.W., Moroney, J.T., Sano, M., Stern, Y. *Incidence of dementia after ischemic stroke. Results of a longitudinal study.* Stroke 2002, 33: 2254-60.

13. Broderick, J.P. William M. *Feinberg Lecture: Stroke therapy in the year 2025. Burden, breakthroughs, and barriers to progress.* Stroke 2004, 35: 205-11.

14. Goldstein, L.B., Adams, R., Becker, K. et al. *Primary prevention of ischemic stroke. A statement for healthcare professionals from the Stroke Council of the American Heart Association.* Stroke 2001, 32: 280-99.

15. Murphy, S. *Production of nitric oxide by glial cells: Regulation and potential roles in the CNS.* Glia 2000, 29: 1-13.

16. Chao, C.C., Hu, S., Sheng, W.S., Bu, D., Bukrinsky, M.I., Peterson, P.K. *Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism.* Glia 1996, 16: 276-84.

17. Hu, J., Castets, F., Guevara, J.L., Van Eldik, L.J. *S100β stimulates inducible nitric oxide synthase activity and mRNA levels in rat cortical astrocytes.* J Biol Chem 1996, 271: 2543-7.

18. Matsui, T., Mori, T., Tateishi, N., Kagamiishi, Y., Satoh, S., Katsube, N., Morikawa, E., Morimoto, T., Ikuta, F., Asano, T. *Astrocytic activation and delayed infarct expansion after permanent focal ischemia in rats. Part I: Enhanced astrocytic synthesis of S-100β in the periinfarct area precedes delayed infarct expansion.* J Cereb Blood Flow Metab 2002, 22: 711-22.

19. Shimoda, T., Tateishi, N., Shintaku, K., Yada, N., Katagi, J., Akiyama, T., Maekawa, H., Shinagawa, R., Kondo, K., Kondo, K. *ONO-2506, a novel astrocyte modulating agent, suppresses the increase of COX-2 and iNOS mRNA expression in cultured astrocytes and ischemic brain.* Soc Neurosci Abst 1998, 24(Part 1): Abst 384.13.

20. Shinagawa, R., Tateishi, N., Shimoda, T., Shintaku, K., Yada, N., Honjo, K., Kagamiishi, Y., Kondo, K. *ONO-2506 ameliorates neurodegeneration through inhibition of reduction of GLT-1 expression.* Soc Neurosci Abst 1998, 24(Part 1): Abst 384.14.

21. Shinagawa, R., Tateishi, N., Shimoda, T., Maekawa, H., Yada, N., Akiyama, T., Matsuda, S., Katsube, N. *Modulating effects of ONO-2506 on astrocytic activation in cultured astrocytes from rat cerebellum.* Soc Neurosci Abst 1999, 25(Part 2): Abst 843.10.

22. Katsumata, S., Tateishi, N., Kagamiishi, Y., Shintaku, K., Hayakawa, T., Shimoda, T., Shinagawa, R., Akiyama, T., Katsube, N. *Inhibitory effect of ONO-2506 on $GABA_A$ receptor disappearance in cultured astrocytes and ischemic brain.* Soc Neurosci Abst 1999, 25(Part 2): Abst 843.11.

23. Matsuda, S., Maekawa, H., Shimoda, T., Shinagawa, R., Akiyama, T., Tateishi, N., Katsube, N. *Effects of ONO-2506 on the glutathione synthesis and hydrogen peroxide toxicity.* 32nd Annu Meet Soc Neurosci (Nov 2-7, Orlando) 2002, Abst 232.12.

24. Tateishi, N., Mori, T., Kagamiishi, Y., Satoh, S., Katsube, N., Morikawa, E., Morimoto, T., Matsui, T., Asano, T. *Astrocytic activation and delayed infarct expansion after permanent focal ischemia in rats. Part II: Suppression of astrocytic activation by a novel agent (R)-(–)-2-propyloctanoic acid (ONO-2506) leads to mitigation of delayed infarct expansion and early improvement of neurologic deficits.* J Cereb Blood Flow Metab 2002, 22: 723-34.

25. Honjo, K., Tateishi, N., Shintaku, K., Satoh, S., Katsube, N. *Effects of ONO-2506 combined with thrombolytic therapy in a rat model of thrombotic focal cerebral ischemia.* Soc Neurosci Abst 2001, 27: Abst 865.5.

26. Honjo, K., Tateishi, N., Shintaku, K., Shinagawa, R., Kagamiishi, Y., Katsube, N. *Delayed increase of extracellular glutamate probably due to reduction of GLT-1 and GLAST following transient focal cerebral ischemia and of its inhibition by ONO-2506.* Soc Neurosci Abst 1999, 25(Part 2): Abst 843.12.

27. Tateishi, N., Shintaku, K., Kitajima, S., Kohjimoto, Y., Li, X.H., Mori, T., Katsube, N. *Effects of ONO-2506, an astrocyte modulating agent, on neurological symptoms after focal ischemia in cynomolgus monkeys.* 32nd Annu Meet Soc Neurosci (Nov 2-7, Orlando) 2002, Abst 489.7.

28. Kato, H., Araki, T., Imai, Y., Takahashi, A., Itoyama, Y. *Astroglia-mediated neuroprotection by ONO-2506 against MPTP neurotoxicity in mice.* Soc Neurosci Abst 2001, 27: Abst 199.3.

29. Yada, N., Shimoda, T., Yamashita, D., Akiyama, T., Katsube, N., Tateishi, N. *Effects of ONO-2506 on amyloidgenic pathway: Implications to the treatment of Alzheimer's disease.* 33rd Annu Meet Soc Neurosci (Nov 8-12, New Orleans) 2003, Abst 526.3.

30. Kishi, A., Yoshida, M., Kagamiishi, Y., Katsube, N., Mori, T., Tateishi, N. *Effects of ONO-2506 on learning impairments in senescence-accelerated mouse (SAMP8).* 33rd Annu Meet Soc Neurosci (Nov 8-12, New Orleans) 2003, Abst 731.2.

31. Pettigrew, L.C., Kasner, S.E., Kwiatkowski, T., Albers, G.W., Gorman, M., Grotta, J.C., Sherman, D., Funakoshi, Y. *Safety and tolerability of ONO-2506 in acute ischemic stroke.* Stroke 2004, 35(1): Abst P208.

32. Pettigrew, L.C., Kasner, S.E., Gorman, M., Atkinson, R.P., Dietrich, D., Ishibahi, H. *ONO-2506 suppresses S-100β in ischemic stroke patients.* Stroke 2004, 35(1): Abst P184.

33. Ono Pharmaceutical Annual Report 2003.

Additional References

Asano, T., Matsui, T., Morikawa, E., Tateishi, N., Kagamiishi, Y., Satoh, S., Kondo, K. *The ameliorative effect of ONO-2506 on the delayed and prolonged expansion of the infarct volume following permanent middle cerebral artery occlusion in rats.* J Cereb Blood Flow Metab 1999, 19(Suppl. 1): Abst 64.

Shintaku, K., Tateishi, N., Kagamiishi, Y., Sato, S., Katsumata, S., Nakayama, Y., Hayakawa, T., Katsube, N. *Ameliorative effects of ONO-2506 on neurological deficits and infarction after transient MCA occlusion in rats: Study of therapeutic time window.* Soc Neurosci Abst 1999, 25(Part 2): Abst 843.9.

Matsui, T., Tateishi, N., Kagamiishi, Y., Satoh, S., Kondo, K., Morikawa, E., Taniguchi, T., Mori, T., Asano, T. *Protective effects of ONO-2506 on neurological deficits and brain infarct volume following 1 week of permanent occlusion of middle cerebral artery in rats.* Soc Neurosci Abst 1998, 24(Part 1): Abst 102.7.

Kagamiishi, Y., Tateishi, N., Shintaku, K., Satoh, S., Katsumata, S., Shinagawa, R., Shimoda, T., Katsube, N., Kondo, K. *Ameliorative effects of ONO-2506 on brain damage following the transient ischemia in rats.* Soc Neurosci Abst 1998, 24(Part 1): Abst 384.11.

Matsuda, S., Maekawa, H., Shimoda, T., Shinagawa, R., Tateishi, N., Katsube, N. *Effect of ONO-2506 on the production/consumption of glutathiones in primary rat cultured cortical astrocytes.* Soc Neurosci Abst 2001, 27: Abst 715.16.