Synthesis and bioevaluation of alkyl 2-cyanoacryloyl glycolates as potential soft tissue adhesives

H. Jaffe,* C. W. R. Wade, A. F. Hegyeli,† R. Rice, and J. Hodge
U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland 21701

A series of alkyl 2-cyanoacryloyl glycolate tissue adhesives were synthesized and characterized by NMR. Physical properties and bond strengths are presented. Within the series, bond strength decreased with increasing molecular weight. Corresponding polymers were evaluated by *in vitro* and *in vivo* techniques for biocompatibility. In general, *in vitro* biocompatibility increased with molecular weight. Based on *in vitro* and *in vivo* results, the isobutyl and isoamyl derivatives gave polymers that were most biocompatible, however, the entire series was found to be less reactive than poly(methyl 2-cyanoacrylate) and only the isopropyl derivative polymers more reactive than poly(isobutyl 2-cyanoacrylate). Approximately one-third of the isobutyl polymer biodegraded *in vivo* after 6 weeks.

INTRODUCTION

The rapidly polymerizing alkyl 2-cyanoacrylates have been used as tissue adhesives and hemostatic agents because of their ability to adhere to moist tissue.[1] In the homologous series of poly(alkyl 2-cyanoacrylates) the lower alkyl homologues, e.g., methyl, exhibit the most rapid rate of biodegradation but are also the most toxic to tissue. The higher alkyl homologues, e.g., isobutyl and isoamyl, which exhibit low tissue toxicity are most frequently used as tissue adhesives, hemostatic agents and antiblister agents, and osseous adhesives. In addition they exhibit good wetting and spreading characteristics on proteinaceous tissue. However, a serious shortcoming of the higher alkyl homologues are their long lifetimes in tissue caused by their low rates of biodegradation.[2–6]

In an effort to find an "ideal" tissue adhesive, our laboratory undertook the synthesis of 2-cyanoacrylate derivatives containing easily hydrolyzable nontoxic groups, such as, glycolate esters and glycerol ketals,[7–9] which could provide a "handle" on the resulting polymer for rapid biodegradation. We

*Present address: USDA, ARS, AEQI, Livestock Insects Laboratory, Rm. 120, Bldg. 307, BARC-East, Beltsville, MD 20705.
†Deceased.

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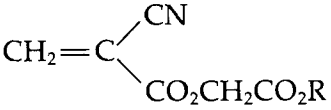
now report the synthesis and preliminary bioevaluation of this new series of potential tissue adhesives, the alkyl 2-cyanoacryloyl glycolates:



R = Me,Et,*i* − Pr,*i* − Bu,*i* − Am

The incorporation of the glycolyl ester group in these molecules provides an additional site for hydrolytic attack and thus should increase the biodegradability of these compounds. The aliphatic alcohols and glycolic acid released upon hydrolysis are expected to be of relatively low tissue toxicity.

MATERIALS AND METHODS

Starting materials

Glycolate esters were purchased (Fisher Scientific, Silver Springs, MD) or synthesized from glycolic acid and the appropriate alcohol. All other reagents were of the highest purity commercially available. Solvents were ACS reagent grade used without further purification.

Alkyl 2-cyanoacetyl glycolates

The alkyl 2-cyanoacetyl glycolates were prepared by Fisher esterification of the glycolate esters and cyanoacetic acid in benzene or by use of dicyclohexylcarbodiimide (DCC) in the THF (see Fig. 1). The resulting alkyl

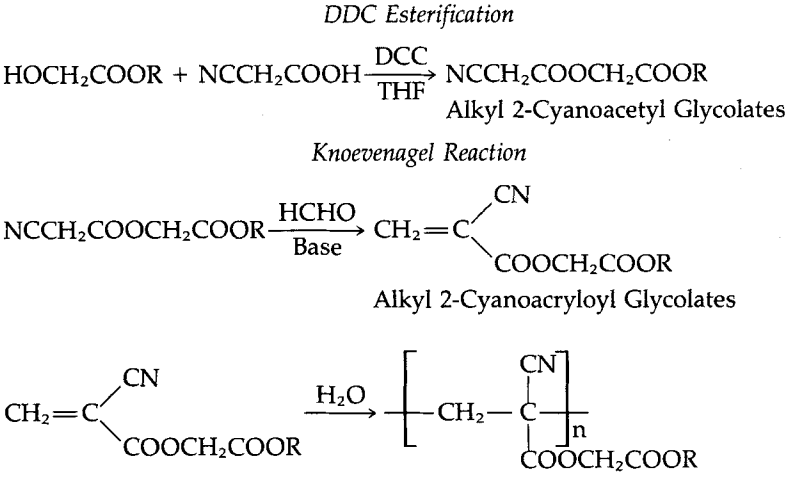


Figure 1. Synthesis of alkyl 2-cyanoacryloyl glycolate monomers and polymers.

2-cyanoacetyl glycolates were purified by fractional distillation. Purity of the fractions was checked by NMR and glpc on 3% OV1. The synthesis of alkyl 2-cyanoacetyl glycolates by DCC esterification is illustrated by the following example for isopropyl 2-cyanoacetyl glycolate.

To a stirred mixture of 94.50 g (0.800 mol) isopropyl glycolate and 68.05 g (0.800 mol) cyanoacetic acid in 1000 mL THF maintained at 5–10°C, was added dropwise a solution of 165.06 g (0.800 mol) dicyclohexylcarbodiimide in 500 mL THF. The resulting thick suspension was allowed to warm up to room temperature, filtered to remove the dicyclohexylurea, and evaporated to give an oil. After cooling overnight, additional urea was filtered off to give crude isopropyl 2-cyanoacetyl glycolate. Fractional distillations at reduced pressure through a short Vigreux column gave (123.4 g, 84%) pure isopropyl 2-cyanoacetyl glycolate, b.p. 93°C/0.10 mm Hg, with infrared and NMR spectra consistent with the proposed structure. Purity was checked by glpc (3% OV1).

Alkyl 2-cyanoacryloyl glycolates

When the pure alkyl cyanoacetyl glycolates were allowed to react with paraformaldehyde under Knoevenagel reaction conditions, the theoretical amount of water was produced in each case. The resulting oligomers were cracked under SO_2 to give alkyl 2-cyanoacryloyl glycolates (Fig. 1). Short path distillation at reduced pressure gave pure materials. Structural confirmation was done by NMR on a model T60A (Varian Associates, Palo Alto, CA) instrument. Physical and NMR data are presented in Table I. The synthesis of alkyl 2-cyanoacryloyl glycolates is illustrated by the following example for isopropyl 2-cyanoacryloyl glycolate.

TABLE I
Physical and NMR Data of Alkyl 2-Cyanoacryloyl Glycolates

Alkyl group	Boiling point (°C)	NMR Data[a]
Methyl	108–12/1.0 mm	($CDCl_3$) δ 3.85 (s, 3, CH_3), 4.90 (s, 2, CH_2CO), 6.97 (s, 1, olefinic H) and 7.32 (s, 1, olefinic H)
Ethyl	118–24/1.35 mm	($CDCl_3$) δ 1.30 (t, 3, CH_3), 4.31 (q, 2, CH_2CH_3), 4.88 (s, 2, CH_2CO), 6.92 (s, 1, olefinic H) and 7.30 (s, 1, olefinic H)
Isopropyl	108–15/0.70 mm	(CCl_4) δ 1.27 (d, 6, $CH(CH_3)_2$, 4.80 (s, 2, CH_2CO), 5.14 (m, 1, $CH(CH_3)_2$), 6.91 (s, 1, olefinic H) and 7.25 (s, 1, olefinic H)
Isobutyl	126/0.60 mm	($CDCl_3$) δ .95 (d, 6, $CH(CH_3)_2$), 1.98 (m, 1, $CH(CH_3)_2$), 4.03 (d, 2, $CH_2CH(CH_3)_2$), 4.90 (s, 2, CH_2CO), 6.88 (s, 1, olefinic H), and 7.28 (s, 1, olefinic H)
Isoamyl	110–16/0.25 mm	($CDCl_3$) δ .93 (d, 6, $CH(CH_3)_2$), 1.31–2.07 (m, 3, CH_2CH), 4.30 (t, 2, OCH_2CH_2), 4.90 (s, 2, CH_2CO), 6.90 (s, 1, olefinic H) and 7.30 (s, 1, olefinic H)

[a]In ppm from TMS.

To a refluxing solution of 37.04 g (0.200 mol) isopropyl 2-cyanoacetyl glycolate and three drops of piperidene in 75 mL benzene, was added 6.00 g (0.200 mol) of paraformaldehyde portionwise over a period of several hours. After 30 h of reflux, 3.6 mL water had been removed by azeotropic distillation into a Dean-Stark trap. Benzene (60 mL) was distilled off and 20 mL tricresyl phosphate, 3.00 g P_2O_5 and 0.30 g pyrogallol added to the pot. The Dean-Stark trap was replaced with an oven-dried, acid-washed short path distillation apparatus with SO_2 bleed. The polymeric pot materials were cracked under SO_2 into a chilled receiver containing a small amount of pyrogallol and P_2O_5 at 135°C at 1–2 mm Hg. The crude monomer (16.82 g, 43%) was fractionally distilled under SO_2 through an oven-dried, acid-washed short path apparatus, b.p. 108–15°/0.70 mm Hg. Prior to redistillation, 10 mL tricresyl phosphate, 1.50 g P_2O_5 and 0.15 g pyrogallol were added to the crude monomer. The purity of the fractions and structure were determined by NMR. The monomer was stored in the cold over a trace of *p*-methoxyphenol.

Poly (alkyl 2-cyanoacryloyl glycolates)

Poly (alkyl 2-cyanoacryloyl glycolates) were prepared from the corresponding monomers by dropwise addition of 10.00 g of monomer to a 400 mL vigorously stirred pH 7.00 buffer. The resulting precipitate was stirred vigorously with water in a blender, triturated with distilled-in-glass hexane and dried *in vacuo* to give polymer as a fine white powder.

Poly(isobutyl 2-cyanoacryloyl glycolate-2-^{14}C)

Isobutyl 2-cyanoacetyl glycolate-2-^{14}C was prepared as described above by DCC esterification of 33.04 g isobutyl glycolate (0.250 mol), 0.0454 g ^{14}C-cyanoacetic acid (0.0227 mCi/mg) and 21.27 g cyanoacetic acid (0.250 mol) in THF. The specific activity of the resulting purified product was determined by liquid scintillation methods to be 27,846 DPM/mg.

Isobutyl 2-cyanoacryloyl-2-^{14}C glycolate monomer and its corresponding polymer were prepared from isobutyl 2-cyanoacetyl-2-^{14}C glycolate as described above. The ^{14}C-polymer had a specific activity of 23,100 DPM/mg.

Bond strengths of the monomers

Bond strengths of the monomers on Aluminum test blocks were determined by ASTM method 0897-49 and are shown in Table II. Ten determinations were made on completely polymerized samples kept for more than 22 h. The value listed in the average of two to four samples.

TABLE II
Bond Strength of Alkyl 2-Cyanoacryloyl Glycolates

Alkyl group	Mean bond strength (psi)
Methyl	2375
Ethyl	1515
Isopropyl	616[a]
Isobutyl	1445
Isoamyl	1238

[a]Samples had not completely polymerized after 119 hrs.

In vitro and *vivo* biocompatibility studies

The alkyl 2-cyanoacryloyl glycolate polymers were screened by our previously described methods for *in vitro* and *in vivo* biocompatibility.[7,8]

In vivo biodegradation

Male rats (Sprague-Dawley derivation), weighing from 200 to 275 g were used as test animals. Approximately 100 mg of labeled isobutyl 2-cyanoacryloyl glycolate was compressed into pellets. The pellets were cut in half and each of the halves accurately weighed and implanted into the rats essentially as described previously.[8] The incisions were closed and the rats were given food and water *ad libitum.*

Two rats each were killed at 2, 4, and 6 weeks. The implants were excised and transferred quantitatively to 10 mL flasks. Tissue dissecting solution (hyamine hydroxide) was added and the mixture was agitated at 60°C until dissolution was complete. The solution, diluted to exactly 10 mL, was used for assay for radioactivity.

RESULTS AND DISCUSSION

The synthetic routes for the preparation of the alkyl 2-cyanoacryloyl glycolates and their polymers are shown in Figure 1. The use of DCC in the esterification step resulted in high yields and simplified purification. Physical and NMR data for these new compounds are listed in Table I. In all cases, chemical shifts were as expected.

Bond strengths are shown in Table II and are comparable to the simple alkyl 2-cyanoacrylates.[10] As expected the bond strength of the methyl derivative was highest and declined throughout the series with increasing molecular weight. All derivatives had bond strength in excess of that needed for tissue adhesives. The anomolous result for the isopropyl derivative is due to incomplete polymerization of the sample probably caused by the presence of excess inhibitor and/or lack of moisture on the test plates.

TABLE III
***In Vitro* and *In Vivo* Toxicity of the Poly(Alkyl 2-Cyanoacryloyl Glycolates) and Poly(Alkyl 2-Cyanoacrylate)**

Alkyl group	*In vitro* class	*In vivo* class	
		7 days	28 days
Me	III	II	I
Et	II	I	II
i-Pr	III	III	II
i-Bu	I	I	II
i-Am	I	II	II
Me[a]	III	III	III
i-Bu[a]	II	II	II

Note: I = *in vitro*, little or no growth inhibition, *in vivo*, slight or no tissue reaction; II = *in vitro*, moderate growth inhibition, *in vivo*, moderate tissue reaction; III = *in vitro*, extensive growth inhibition, *in vivo* – severe tissue reaction.
[a]Alkyl 2-Cyanoacrylates from ref. 8–9.

Results of the *in vitro* and *in vivo* biocompatibility studies of the polymers are shown in Table III. Also shown are data on the poly(methyl and isobutyl 2-cyanoacrylate) for comparison. At the time of kill all of the rats appeared to be healthy and showed no obvious signs of illness. There was no gross evidence of inflammation at the site of implantation and the implants were cleanly and easily removed from the muscle tissue. Recovery of the remaining implant appeared to be total, indicating that no losses occurred because of the handling and removal of the implant.

As seen in Table III, *in vitro* toxicity of the alkyl 2-cyanoacryloyl polymers appears to generally decrease with increasing molecular weight and with the expected decreasing rate of biodegradation. Based on the *in vitro* and *in vivo* results, the isobutyl and isoamyl derivatives are the most biocompatible, however, the entire series as a group was found to be less reactive than poly(methyl 2-cyanoacrylate) and all except the isopropyl less reactive than poly(isobutyl 2-cyanoacrylate). The biocompatibility screening of the series did not eliminate any of the compounds from further evalution, except, perhaps the isopropyl derivative because it was most reactive *in vivo* and in the toxic class *in vitro*.

The methyl derivative, even though in the *in vitro* toxic class, would be a good candidate for further study because of its superior bond strength, predicted rapid biodegradation rate and similar tissue reaction to the widely used isobutyl 2-cyanoacrylate. Some evidence for an initial rapid biodegradation of the methyl derivative can be seen by its toxic classification *in vitro* and a decline of its *in vivo* toxicity classification from 7 days (group II) to 28 days (group I). A similar effect was observed[8,9] with the rapidly biodegradable methyl 2-cyanoacrylate.

Our radiobiodegradation experiment indicated that approximately two-thirds of the isobutyl derivative remained after 6 weeks which indicates a

somewhat more rapid biodegradation rate than the alkyl 2-cyanoacrylate series. Based on this limited data it would be premature to draw extensive correlation between degradation rates and toxicity data, though it does seem likely that the lower expected degradation rates of isoamyl and isobutyl compounds compared to the other derivatives can be correlated with their greater biocompatibility. The radiobiodegradation data presented is intended to be preliminary, but in light of past difficulties in attempting to bring the cyanoacrylate tissue into clinical use in the United States, further work on these compounds, including a complete radiobiology absorption study, is clearly indicated.

In conclusion, our results demonstrate that incorporation of the alkyl glycolyl ester in place of an alkyl group does not significantly alter bonding properties of the adhesives. All of the adhesives had bonding strength that is greater than required to effectively hold tissue together. The new materials are biodegradable, relatively non toxic and worthy of further long-term study as tissue adhesives.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council. The opinions or assertations contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

This article is dedicated to the memory of our friend and colleague Andrew F. Hegyeli.

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