Table II. Neuromuscular Activity of Germine Compounds

Compd[a]	IV dose,[b] mg/kg	N[c]	P/Po[d] Before dosing	P/Po[d] After dosing	Δ[e]	Rel potency[f]
1a	20	4	0.19 (0.08)	0.55 (0.15)	0.36	~0.05
1d	5[g]					
1b	40	4	0.29 (0.09)	0.75 (0.07)	0.46	~0.025
1c	1	4	0.20 (0.05)	0.70 (0.18)	0.50	1
1e	1	3	0.41 (0.08)	0.92 (0.28)	0.51	1

[a]See Table I. [b]Compounds dissolved in water and solution adjusted to pH 5 with HCl. [c]Number of cats per dose. [d]Ratio of twitch tension (*P*) to tetanic tension (*Po*) of gastrocnemius–soleus muscle in anesthetized cats; average value reported for tests with standard deviation given in parentheses. [e]Difference of average tension responses before and after dosing. [f]**1c** (germine 3-acetate) = 1. [g]Insufficient material for dose–response test; compound inactive at 1.0 and 5.0 mg/kg iv dose.

work in the germine series.[4] As a control, a mixture rich in all of the germine 3,16-diacetate degradation components was subject to treatment with periodate, and only the new diacetate proved to be inert. The only possible diacetate consistent with acylation to tetraacetate **1f**, yet inert to periodate, thus, lacking a vicinal diol system, is germine 3,15-diacetate (**1e**). The amorphous diester was converted to its crystalline oxalate salt for analysis and testing.

As previously noted[2] the second unknown component formed in the degradation of germine 3,16-diacetate (**1b**) appeared to be a monoacetate by tlc R_f. It is also generated from germine 16-acetate.[2] We have shown by comparative tlc that it is also generated *via* the degradation of germine 3,15-diacetate (**1e**) in aqueous solution. This suggests that the final unknown component formed in the aqueous degradation of **1b**, **1d**, and **1e** is germine 15-acetate (**1g**). No effort has been made to isolate and further characterize this compound (Table I).

The 16 to 15 transacylation which occurs in **1b** and probably in **1d** has proven to be an unusually facile reaction. Qualitatively it occurs at a rate comparable to the hydrolysis at 16, which is known to be intramolecularly catalyzed by the nitrogen present in the EF ring juncture.[5] In nonaqueous systems (methylene chloride, chloroform, acetonitrile, acetone, and pyridine) **1b** is stable,[2] and only in the presence of water does the transacylation occur. Presently, we see no strong driving force which explains the facility of the isomerization. However, there is sufficient flexibility in the ring system to permit interaction between positions 15 and 16.

Biological Data. The compound was tested relative to its ability to affect tension responses of the gastrocnemius and soleus muscles to stimulation of the sciatic nerve in anesthetized cats.[6] For comparison, data on all of the discussed germine esters are reported. Germine 3,15-diacetate was shown to be comparable in activity to germine 3-acetate under conditions in which negligible degradation had occurred. Both the 3-acetate and the 3,15-diacetate are 40 times as active as germine 3,16-diacetate. The data are reported in Table II.

Experimental Section

All the work was done using silica gel plates employing a freshly prepared solution of ethyl acetate–methanol–concentrated ammonia (80:15:5) for elution.[2] The approximate R_f's of the six components formed in the degradation of **1b** are: germine 3,15-diacetate (**1e**), 0.80; germine 3,16-diacetate (**1b**), 0.75; germine 3-acetate (**1c**), 0.60; germine 15-acetate (**1g**), 0.45; germine 16-acetate (**1d**), 0.40; germine, 0.25.

The periodate experiments were performed as previously described[4] and analyzed by tlc as indicated above. The acylation to form the tetraacetate from germine 3,15-diacetate was run as described by Kupchan[3] for the preparation from germine, and a 75% yield of **1b** was obtained, identical with authentic material prepared from germine.

Germine 3,15-Diacetate (1e). A solution of 10 g of germine 3,16-diacetate (**1b**) in 100 ml of pyridine and 100 ml of water was allowed to stand for 3 weeks at room temperature. The reaction solution was monitored by tlc as indicated above. After the 3-week period most of the germine 3,16-diacetate had degraded, but substantial germine 3,15-diacetate remained. After addition of 50 ml of concentrated NH_4OH the reaction solution was extracted with 3 × 100 ml of $CHCl_3$, which was dried over $MgSO_4$, filtered, concentrated at reduced pressure, and dried at 100° (vacuum) to afford 8.1 g of a colorless glass. Tlc examination (as above) indicated the presence of germine 3,15-diacetate, germine 3-acetate, and germine as the principal components. Dry column chromatography of 1 g of the product mixture on 200 g of Brinkmann silica gel H using 10% EtOH in $CHCl_3$ as the eluent, and taking 10-ml fractions, afforded essentially single-spot germine 3,15-diacetate in fractions 91–150. These were combined and concentrated to afford 0.14 g of **1b** as an amorphous powder whose chemical and spectral properties were as described in the text. Repeated attempts to crystallize the free base were not successful. To this material in methanol was added 1 molar equiv of oxalic acid. Concentration and trituration of the residual gum with CH_3CN afforded 139 mg of germine 3,15-diacetate oxalate monohydrate: mp 214–216° dec. *Anal.* ($C_{33}H_{15}NO_{15}$) C, H, N.

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Polymeric Salicylate Derivatives

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The attachment of biologically active compounds to polymers has been recently investigated as a means of increasing their duration of activity.[1-12] In the present work we have studied the attachment of salicylates, which are important as analgetics and antiinflammatory agents by carbonate linkages to starch. These linkages can suffer chemical and enzymatic hydrolysis releasing the active compound in the body.

Some work on the incorporation of salicylates into polymers has been carried out.[13-19] Thus, *O*-acetylsalicyloyl chloride was allowed to react with soluble starch in the presence of potassium hydroxide, and a polymer having 0.7 aspirin groups per glucose unit was obtained.[13-14]

Table I. Some Data Concerning Salicylates Bound to Starch[a]

Polymer	Salicylate, g (mol)	Et_3N, mol	$COCl_2$, mol	Polymer yield, g	Methoxyl, %	Carbon, %	D.S.[b]	% salicylate ester in polymer	Anhydroglucose units per salicylate residue	Dicarbonate, g (yield, %)
1	5 (0.033)	0.033	0.049	3.8	8.3		0.82	43.5	1	1.7 (31)
2	3.5 (0.025)	0.025	0.037	2.42		49.1	0.14	15.8	7	2.0 (53)
3	7.9 (0.037)	0.037	0.056	3.75		56.7	0.61	37.8	2	3.8 (34)

[a]Starch (2 g, 0.0124 mol, anhydroglucose units) in pyridine was allowed to react with the chloroformate derivative of the salicylates obtained by reaction of the salicylate ester with phosgene in toluene (12.5% solution) in the presence of triethylamine. The salicylate polymer was isolated as well as the salicylate dicarbonate obtained as a side product. [b]The D.S. (degree of substitution per glucose unit) of the methyl salicylate polymers was calculated from methoxyl analysis, while that of the phenyl salicylate polymers was calculated from carbon analyses.

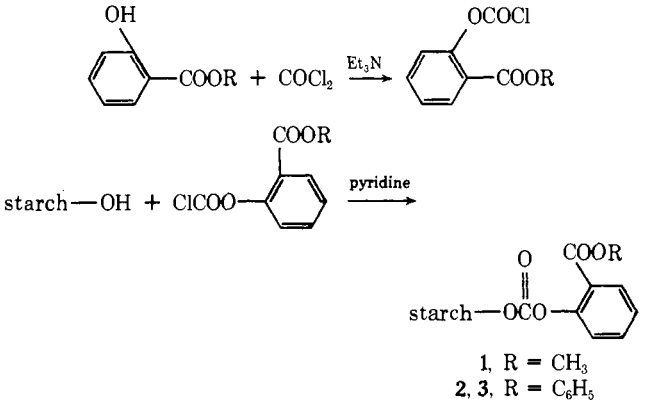
Table II. Antiinflammatory Action of Polymers **1–3**

Polymer	Differences between the paws at hour (units)[a]							
	1	2	3	4	6	8	10	20
1	5.19	5.82	8.50	9.50	7.00	6.00	5.20	2.82
2	4.50	7.08	6.78	6.50	7.08	5.60	4.50	2.30
3	3.30	7.00	6.60	5.60	5.50	3.00	3.20	1.00
Control	6.00	6.30	8.70	9.30	8.04	6.00	4.83	4.01
Aspirin	3.00	3.10	4.00	5.03	6.30	6.00	4.50	3.92

[a]Volume displacement units, as measured by Volume plethysmographic meter (Ugo Basili, Milan, Italy).

However, its duration of activity was short. In the same manner *O*-acetylsalicylic acid was attached to polyvinyl alcohol. The polymer contained up to 3% ester groups.[15] It showed the same type of activity as aspirin, but it was less toxic. Polymers containing salicylic acid derivatives were prepared by esterification of the free phenolic groups with methacryloyl chloride and polymerization of the methacrylate ester formed.[20]

In the present work the chloroformate derivatives of methyl and phenyl salicylate were prepared *in situ* by reaction with phosgene in the presence of triethylamine, and these were allowed to react with soluble starch in pyridine. Different degrees of substitution were achieved depending on the concentration of the reactants (Table I).



1, R = CH_3
2, 3, R = C_6H_5

Dimethyl salicylate carbonate and diphenyl salicylate carbonate were isolated as side products and were identified by elemental analysis, nmr spectra, mass spectral data, and ir spectra. The latter carbonate seems not to have been reported before.

Preliminary Biological Evaluation. The antiinflammatory activity of the following polymers was investigated and was compared to that of aspirin (Table II): starch substituted with methyl salicylate (D.S. = 0.82, *i.e.*, 0.82 salicylate group per anhydroglucose unit) (**1**); starch substituted with phenyl salicylate (D.S. = 0.14) (**2**); starch substituted with phenyl salicylate (D.S. = 0.61) (**3**).

All the above-mentioned polymers, especially **3**, exhibit longer duration of activity than aspirin. Polymer **3** was still active after 20 hr, while aspirin's activity ceased much sooner (Table II).

In conclusion, the attachment of salicylate derivatives to starch by carbonate linkages increased their duration of activity. This may be due to a slow release of the drug *via* hydrolysis, but the possibility that the polymeric drug is acting as a whole cannot be excluded.[14]

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Nmr spectra were taken on a Varian T-60 instrument, and ir spectra on a Perkin-Elmer Model 257 instrument. Where analytical results are indicated only by the symbols of the elements, the observed values differed from the calculated values by not more than ±0.4%.

Salicylate–Starch Polymers (1–3). A solution of the salicylate ester and triethylamine was added dropwise during 30 min to a stirred solution of 12.5% phosgene in toluene at −10°. Stirring was continued for another 40 min in the cold and for an additional hour at room temperature. Excess phosgene was evaporated *in vacuo* and the precipitate of triethylamine hydrochloride was filtered. The residue containing the chloroformate derivative was concentrated *in vacuo* at 50° to 15–20 ml.

Soluble starch (Analar, BDH) (containing 15% H_2O, molecular weight 18,000) in 10 ml of pyridine–water solution was heated until it dissolved. To the clear solution pyridine (80 ml) was added. The mixture was mechanically stirred while the pyridine was slowly distilled off until the boiling point of the distillate reached 115°. The resulting suspension of dry starch in pyridine (~40 ml) was cooled to 0° and the solution of the chloroformate prepared above was added dropwise with stirring during 30 min. Stirring was continued for another 12 hr at room temperature. The solid polymer was filtered and washed with ether and ethanol: ir (Nujol) 3450 (OH), 1770 (OCOO), 1740 ($COOC_6H_5$), 1030 cm^{-1} (α-glucoside linkage).

Chloroform (250 ml) was added to the filtrate followed by 0.1 *N* HCl (150 ml). The organic phase was separated and washed with water. It was dried and evaporated *in vacuo*. Petroleum ether (200 ml) was added to the residue, and the solid disalicyloyl carbonate that separated was washed with ethanol and recrystallized from chloroform–petroleum ether: ir data for diphenyl sali-

cyloyl carbonate (Nujol) 1795 (OCOO), 1740 cm^{-1} ($COOC_6H_5$); nmr ($CDCl_3$) δ 7.0–7.9 (m, 18, C_6H_4, C_6H_5); mp 130°; mass spectrum *m/e* (rel intensity) 362 (59), 361 (83), 242 (59), 241 (100), 197 (34), 196 (83), 121 (51), 120 (63), 92 (57). *Anal.* ($C_{27}H_{18}O_7$) C, H.

The antiinflammatory tests were carried out as follows. Male albino rats (6–8 per group) weighing 150–160 g were used. The polymers were made up as suspensions in Tragacanth. The control vehicle was 1 drop of DMF diluted by the same factor. This itself does not cause any effect. Edema in one leg was obtained by an injection of carrageenin in the usual method. After 1 hr, when it was ensured that all animals had a well-developed inflammation, the compounds were injected subcutaneously into the neckscruff in 1 ml/kg doses. The control group had carrageenin alone with 1 ml/kg sc of the vehicle, while a positive control was considered to be a relatively high dose of aspirin (200 mg/kg). All compounds were given as 200 mg/kg doses, after this was found to be well tolerated in all compounds. After injection of the compounds, differences in the paws were measured every hour for 4 hr and then again at 6, 8, 10, and 20 hr.

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Immunosuppressive and Antiinflammatory Activities of Cinanserin and Its Analogs[1]

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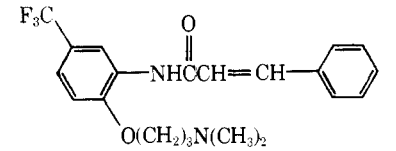
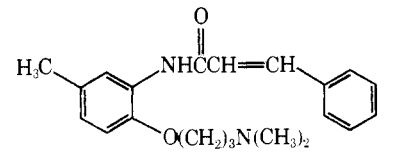
Cinanserin, 2′-(3-dimethylaminopropylthio)cinnamanilide, has been shown to be a potent antagonist of serotonin (Krapcho, *et al.*,[2] Rubin, *et al.*,[3] Furgiuele, *et al.*[4]) that also exhibits analgesic activity (Rubin, *et al.*[5]). Preliminary studies in our laboratory showed that it also possessed immunosuppressive activity. Subsequent studies showed cinanserin to be more active than azathioprine in suppressing the uptake of ^{14}C-labeled leucine and thymidine by phytohemagglutinin (PHA)-stimulated human lymphocytes, in prolonging the survival time of skin grafts between congenic strains of mice differing at the H-2 locus (Schwartz, *et al.*[6]), and in protecting rats against paralysis in experimental allergic encephalomyelitis (Babington and Wedeking[7]). About 100 analogs of cinanserin have been synthesized and evaluated for immunosuppressive and antiserotonin activities. Although most compounds of these series showed both immunosuppressive and antiserotonin activities, and of similar degree, several members exhibited a marked separation of these activities (Krapcho, *et al.*[8]). The immunosuppressive activity of cinanserin was further evaluated and the antiinflammatory activities of several analogs of cinanserin were compared with those of cinanserin, azathioprine, and indomethacin.

Chart I. Chemical Structure of Cinanserin, 2′-(3-Dimethylaminopropoxy)-5′-trifluoromethylcinnamanilide (I), 2′-(3-Dimethylaminopropoxy)-5′-methylcinnamanilide (II), 5′-*tert*-Butyl-2′-(3-dimethylaminopropoxy)cinnamanilide (III), and 2-Chloro-2′-(3-dimethylaminopropoxy)cinnamanilide (IV)[a]



I



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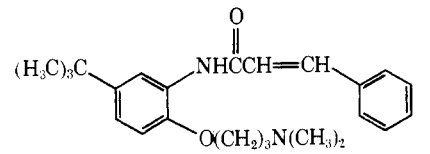
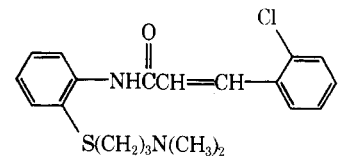
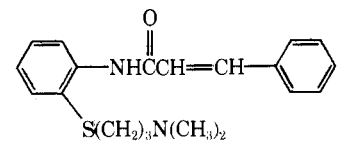


III



IV



cinanserin

[a] With the exception of III, the preparation of the above compounds was reported in ref 8. Compound III (mp 199–201°, crystallized from isopropyl alcohol) was synthesized by C. F. Turk of these laboratories from 4-*tert*-butyl-2-nitrophenol according to the procedure used for the preparation of II.[8]

Experimental Section

Immunosuppressive Activity of Cinanserin. Primary Immune Response. Hemagglutination. The effect of cinanserin on