these data it is clear that a structural dependence exists in the convulsant activity of these lactones and even though each compound contains the picrotoxane skeleton[1] there is sufficient structural diversity to comment on their biochemorphology. Each high activity compound has a lactone function connecting C-3 and -5 of the skeleton. The carbonyl system is *cis* to the fused ring structure and, in combination with the bridgehead hydroxyl at C-6, appears to comprise the absolute structural requirements for activity. In almost every case where either the hydroxyl is protected, *e.g.*, picrotoxinin acetate, or where the lactone system joining C-3 and -5 is absent, *e.g.*, methyl α-picrotoxinate, there is no activity. There is one exception to this generalization and that is α-dihydropicrotoxinin acetate, a compound hydrolyzed to α-dihydropicrotoxinin with great ease. In our view the activity of this compound is due to its hydrolysis product.

From the structures and high activity of picrotoxinin, tutin, and coriamyrtin it appears that oxirane ring location and even perhaps its presence have no role in the biological effect. There also appears to be no requirement for the lactone ring connecting C-2 and -13 in picrotoxinin since the other naturally occurring compounds, all possessing equal activity, do not contain this group.

The substitution pattern at C-4 appears to have a role in determining whether compounds with the requisite lactone and hydroxyl groups are highly active. All the compounds with CD_{50} values at about 1.5 mg/kg have an isopropenyl group *trans* to the lactone and *cis* to the bridgehead hydroxyl. Interaction between the π electrons of the double bond and the proton of the hydroxyl has been well established.[7,8] In α-dihydropicrotoxinin this interaction is precluded and, while that compound is highly active, it has potency about one-fifth that of the parent. Picrotin, containing a hydroxyl group at C-12 instead of hydrogen as in dihydropicrotoxinin, has low activity, a greater steric requirement, and no π-electron interaction with its bridgehead hydroxyl. The indications are that hydrogen bonding of the bridgehead hydroxyl and the π electrons of the isopropenyl group are involved in determining receptor affinity and that C-4 is bulk sensitive to substituents which inhibit access to the lactone system. This is seen in the inactivity of neopicrotoxinin. This compound should be active if the sole activity requirements were the lactone and hydroxyl groups. However, its isopropylidine group is not axial but in the plane of picrotoxane carbons 3, 4, and 5. This yields a greater steric requirement, interrupts the π-electron–hydroxyl group interaction of picrotoxinin, and deshields the hydroxyl.

Additionally, tutin has a free hydroxyl substituent at C-4 of the picrotoxane skeleton. This hydroxyl is *cis* to the lactone and in such close proximity that interconversion of the lactone from a five- to a six-membered ring is possible. On esterification the free OH is converted to the much larger acetoxy group. This could, and apparently does, partially impede approach of the lactone to the concerned receptor since the activity of tutin monoacetate is intermediate.

Although these compounds give an insight into lactone analeptic biochemorphology, they do not permit assessment of bridgehead methyl group importance or that of the fused-ring system. To that end appropriate lactones of various cyclohexanecarboxylic acids are under investigation. Preliminary experiments indicate that simple compounds containing the lactone and hydroxyl groups in the axial–axial arrangement are active.

Acknowledgments.—This work was supported by research Grant MH-08348 and Training Grant 2T 1 GM 461 from the National Institutes of Health, U. S. Public Health Service. The authors are indebted to Mr. C. James Rosene for proton magnetic resonance spectra of the compounds; to Dr. Jiancarlo Jommi, Organic Chemistry Institute, University of Milan, Milan, Italy, for information on the biological activity of hyenancin, isohyenancin, and capenecin; to Dr. Takuo Okuda, Kyoto University, Kyoto, Japan, for a sample of coriamyrtin; and to Dr. W. E. Harvey, Victoria University of Wellington, Wellington, New Zealand, for a sample of tutin.

(7) D. Mercer and A. Robertson, *J. Chem. Soc.*, 288 (1936).
(8) H. Conroy, *J. Am. Chem. Soc.*, **74**, 491 (1952).

Some 4(1H)-Quinolylidene and 1,8-Naphthyrid-4(1H)-ylidene Compounds

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The synthesis and antiinflammatory activity of 4-quinolylacetamide, 7-methyl-1,8-naphthyrid-4-ylacetamide, some novel 4(1H)-quinolylideneacetamides and 1,8-naphthyrid-4(1H)-ylideneacetamides, and also three 2,3,4,6-tetrahydropyrido[3,4-*c*]quinoline-2,4-diones are reported. The most active compound of the series was found to be 1-ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylideneacetamide.

Since the search for new antiinflammatory compounds began in these laboratories, a large number of heterocyclic carboxylic acids and many of their derivatives have been synthesized and screened for biological activity. Among these were the substituted anilinopyridinecarboxylic acids reported by Evans, *et al.*[1]

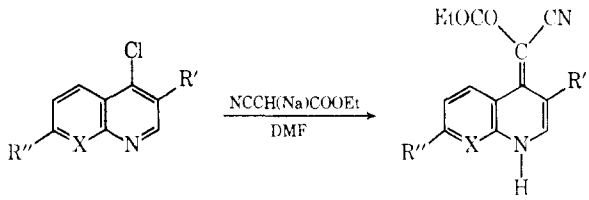
In connection with this program it recently became of interest to prepare a number of substituted acetamides and related compounds.

Chemistry.—Borror and Haeberer[2] reported the reaction between 2-chloroquinoline and sodium ethyl cyanoacetate to give ethyl cyano-2(1H)-quinolylidene-

(1) D. Evans, K. S. Hallwood, C. H. Cashin, and H. Jackson, *J. Med. Chem.*, **10**, 428 (1967).

(2) A. L. Borror and A. F. Haeberer, *J. Org. Chem.*, **30**, 243 (1965).

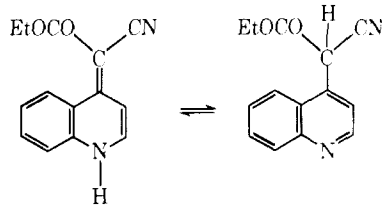
acetate. The latter on hydrolysis with 70% H_2SO_4 gave 2-quinolylacetamide.



1, X = CH; R′ = H; R″ = H
2, X = CH; R′ = CO_2Et; R″ = H
3, X = N; R′ = H; R″ = CH_3

It was found that 4-chloroquinolines and 4-chloro-7-methyl-1,8-naphthyridine reacted in a similar manner yielding the respective 4(1H) analog. Examination of the compounds by uv and visible spectroscopy showed extended conjugation, in comparison with simple quinoline and 1,8-naphthyridine derivatives (see Table I).

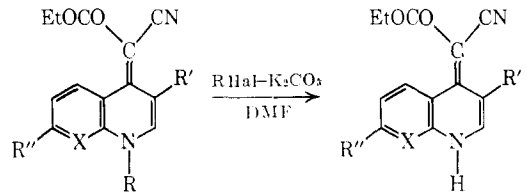
A comparison of the ir spectra of the 4(1H)-naphthyridylidene derivative **3** with the 4(1H)-quinolylidene derivative **1**, in both the solid state (KBr) and in solution ($CHCl_3$), showed certain differences. In KBr disks the spectra were similar, the compounds showing conjugated C≡N absorption at 2180 and 2170 cm^{-1} and C=O absorption at 1680 and 1670 cm^{-1}, respectively. In chloroform solution, however, whereas the spectrum of **3** was virtually identical with that taken in the solid state, **1** showed two C=O bands at 1675 (strong) and 1750 cm^{-1} (medium) indicating the existence of a mixture of the tautomeric forms A and B.



The difference may be accounted for by postulating that in the 4(1H)-quinolylidene **1**, molecules are so arranged in the crystal lattice that intramolecular hydrogen bonding occurs. In solution, the resonance energy of the quinoline ring facilitates the prototropic rearrangement to form B. The resonance energy of the naphthyridine ring appears to be insufficient to effect this rearrangement.

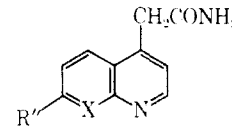
The ir spectra of **2** showed C=O absorption at 1715 (3-COOEt) and 1680 cm^{-1} in the solid state, and at 1715 and 1750 cm^{-1} in $CHCl_3$ solution.

Reaction of the cyanoacetate derivatives **1**, **2**, or **3** with an alkyl halide and anhydrous K_2CO_3 in DMF yielded the corresponding N-alkyl derivatives in fair to good yields. The structure of these derivatives was confirmed by their uv and visible spectra (see Table I) and by their ir spectra which showed conjugated nitrile absorption at 2160–2180 cm^{-1} and C=O absorption at 1650–1680 cm^{-1}, in both the solid state and in solution.

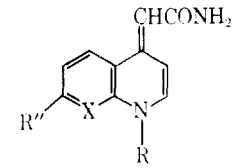


No attempt was made to differentiate between stereoisomers occurring about the exocyclic double bond.

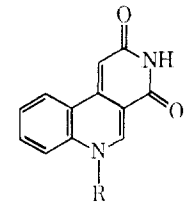
Hydrolysis of the substituted cyanoacetates with 70% v/v H_2SO_4 yielded: (1) from **1** and **3** the respective substituted acetamides (colorless) which showed



normal amide carbonyl absorption at 1670 cm^{-1}; (2) from N-alkyl derivatives of **1** and **3** the corresponding 4(1H)-ylideneacetamides (highly colored) which showed



C=O absorption at 1640 cm^{-1}; and (3) from **2** and its N-alkyl derivatives the 2,3,4,(6)-tetrahydropyrido-[3,4-*c*]quinoline-2,4-diones. All three compounds were



colored and showed absorption maxima in the region of 390–410 mμ in their uv and visible spectra. The C=O absorption in the ir spectrum of **2a** (R = H) was somewhat obscured in KBr, there being strong absorption between 1500 and 1700 cm^{-1}. Compounds **6a** (R = CH_3) and **7a** (R = C_2H_5) showed peak carbonyl absorption at 1660 cm^{-1}. In DMSO all three compounds showed C=O absorption at 1660 cm^{-1}.

Experimental Section

All melting points were determined using a capillary melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. With the exception of 4-chloro-7-methyl-1,8-naphthyridine, 3-carbethoxy-4-chloroquinoline, and **1a** in Table II, all of the compounds are new.

4-Chloro-7-methyl-1,8-naphthyridine was prepared by the method of Brown.[3]

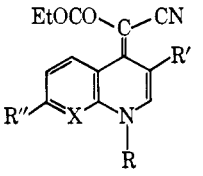
3-Carbethoxy-4-chloroquinoline.—Using a similar method to that described by Kaslow and Clark,[4] 3-carbethoxy-4-hydroxyquinoline (60 g) and $POCl_3$ (150 ml) were heated at reflux temperature for 2 hr. The mixture was poured onto crushed ice and neutralized with NH_4OH and the product was extracted ($CHCl_3$). The organic phase was washed (H_2O) and dried ($MgSO_4$) and the solvent was removed in a rotary evaporator. The residue was recrystallized from petroleum ether (40–60°) to afford colorless needles, 51.3 g (79%), mp 45°. *Anal.* ($C_{12}H_{10}ClNO_2$) C, H, N.

Ethyl Cyano(7-methyl-1,8-naphthyrid-4(1H)-ylidene)acetate (3).—Using a procedure similar to that described by Borror and Haeberer,[1] the sodium salt prepared from ethyl cyanoacetate (19.1 g, 0.169 mole) and NaH (4.45 g, 0.185 mole) was treated with 4-chloro-7-methyl-1,8-naphthyridin (15.0 g, 0.084 mole) in

(3) E. V. Brown, *J. Org. Chem.*, **30**, 1607 (1965).
(4) C. E. Kaslow and W. R. Clark, *ibid.*, **18**, 55 (1953).
(5) F. Zymalkowski and W. Schauer, *Arch. Pharm.*, **290**, 218 (1957).

Table I

Substituted Ethyl Cyano-4(1H)-quinolylideneacetates and Ethyl Cyano-1,8-naphthyrid-4(1H)-ylideneacetates

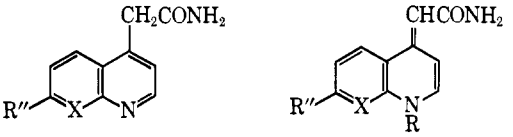


No.	X	R	R′	R″	Recrystn solvent[a]	Yield, %	Mp, °C	Formula[e]	λ_{max}^{MeOH}, mμ	Log ε	Antiinflam act.[b] Carrageenin[c]	Antiinflam act.[b] Uv[d]
1	CH	H	H	H	CF	52	190–192	$C_{14}H_{12}N_2O_2$	420	4.504	39.6	12.6
2	CH	H	CO_2Et	H	AG	62.5	119–121	$C_{17}H_{16}N_2O_4$	422	3.978	Nil	—
3	N	H	H	CH_3	E	81	214–216	$C_{14}H_{13}N_3O_2$	429	4.369	Nil	—
4	CH	C_2H_5	H	H	E	42	154–156	$C_{16}H_{16}N_2O_2$	428	4.564	Nil	—
5	CH	*i*-C_4H_9	H	H	CF	42.5	159–160	$C_{18}H_{20}N_2O_2$	428	4.542	Nil	—
6	CH	CH_3	CO_2Et	H	DE	72	206–208	$C_{18}H_{18}N_2O_4$	426	4.215	Nil	—
7	CH	C_2H_5	CO_2Et	H	E	70	179–182	$C_{19}H_{20}N_2O_4$	426	4.215	Nil	—
8	N	CH_3	H	CH_3	E	52	191–193	$C_{15}H_{15}N_3O_2$	437	4.456	Nil	—
9	N	C_2H_5	H	CH_3	E	79	182–183	$C_{16}H_{17}N_3O_2$	437	4.474	Nil	—
10	N	*n*-C_3H_7	H	CH_3	E	73	187–190	$C_{17}H_{19}N_3O_2$	438	4.467	—	—
11	N	*n*-C_4H_9	H	CH_3	E	76	145–147	$C_{18}H_{21}N_3O_2$	438	4.468	—	—
12	N	*i*-C_4H_9	H	CH_3	E	76	138–139	$C_{18}H_{21}N_3O_2$	438	4.474	—	—
13	N	4-$O_2NC_6H_4CH_2$	H	CH_3	D	78	275–276	$C_{21}H_{18}N_4O_4$	...	...	—	—

[a] A, Me_2CO; B, C_6H_6; C, AcOH; D, DMF; E, EtOH; F, H_2O; G, hexane; J, MeOH; K, EtOAc. [b] Figures represent the per cent inhibition in groups of animals treated with the test compound compared to control. — = not tested. [c] Doses of 100 mg/kg were given orally at 0.5 hr and 3 hr prior to the carrageenin. [d] A single dose of 100 mg/kg was given orally 0.5 hr before exposure to uv light. [e] All compounds showed a correct analysis for C, H, N.

Table II

Substituted Acetamides

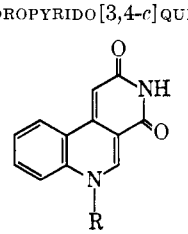


No.	X	R	R″	Recrystn solvent[a]	Yield, %	Mp, °C	Formula[h]	λ_{max}^{MeOH}, mμ	Log ε	Antiinflam act.[b] Carrageenin[c]	Antiinflam act.[b] Uv erythema[d]
1a	CH	...	H	EF	72.5	212–214[g]	$C_{11}H_{10}N_2O$	...	...	59.5	Nil
3a	N	...	CH_3	F	29	190–192	$C_{11}H_{11}N_3O$	315	3.863	Nil	—
4a	CH	C_2H_5	H	E	28	>155[f]	$C_{13}H_{14}N_2O$	410	4.173	Nil	—
5a	CH	*i*-C_4H_9	H	FJ	72	>170[f]	$C_{15}H_{18}N_2O$	410	4.301	Nil	—
8a	N	CH_3	CH_3	J	52	>175[f]	$C_{12}H_{13}N_3O$	427	4.283	14.8	—
9a	N	C_2H_5	CH_3	E	54	>180[f]	$C_{13}H_{15}N_3O$	429	4.310	65.3	55.6
10a	N	*n*-C_3H_7	CH_3	E	40	>150[f]	$C_{14}H_{17}N_3O$	427	4.297	13.6	—
11a	N	*n*-C_4H_9	CH_3	EK	45	>170[f]	$C_{15}H_{19}N_3O$	428	4.330	13.8	—
12a	N	*i*-C_4H_9	CH_3	E	41	>190[f]	$C_{15}H_{19}N_3O$	427	4.310	35.6	18.2
13a	N	4-$O_2NC_6H_4CH_2$	CH_3	A	31	192–194[g]	$C_{18}H_{16}N_4O_3$	423	4.336	47.1	Nil

[a–d] See corresponding footnotes in Table I. [e] Reference 5. [f] The compound decomposes above the indicated temperature. [g] Melts and decomposes. [h] All compounds showed a correct analysis for C, H, N.

Table III

2,3,4,(6)-Tetrahydropyrido[3,4-*c*]quinoline-2,4-diones



No.	R	Recrystn solvent[a]	Yield, %	Mp, °C	Formula[f]	λ_{max}^{MeOH}, mμ	Log ε	Antiinflam act.[b] Carrageenin[c]	Antiinflam act.[b] Uv erythema[d]
2a	H	D	22	>300	$C_{12}H_8N_2O_2$	394	4.230	Nil	—
6a	CH_3	D	27	>300	$C_{13}H_{10}N_2O_2$	404	4.358	Nil	—
7a	C_2H_5	D	51	294–297[e]	$C_{14}H_{12}N_2O_2$	402	4.377	34.8	11.2

[a–d] See corresponding footnotes in Table I. [e] The compound decomposes above this temperature range. [f] All compounds showed a correct analysis for C, H, N.

anhydrous DMF (150 ml). The bulk of the solvent was removed in a rotary evaporator and the residue was dissolved in cold H_2O (1 l.). The product was precipitated by the addition of AcOH and recrystallized (EtOH) to give bright orange crystals, 17.46 g (81%), mp 214–216°.

Ethyl Cyano(1-ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylidene)-acetate (9).—Compound **3** (9.0 g, 0.035 mole), EtI (22.0 g, 0.14 mole), anhydrous K_2CO_3 (10.9 g, 0.08 mole), and anhydrous DMF (75 ml) were boiled under reflux for 6 hr. The bulk of the solvent was removed in a rotary evaporator and the residue was diluted with H_2O (100 ml). The product was recovered by filtration and washed with ice-cold EtOH, followed by H_2O. Recrystallization from EtOH gave bright yellow-green crystals, 7.8 g (79%), mp 182–183°.

The compounds listed in Table I were prepared by procedures similar to those described in the previous two examples.

1-Ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylideneacetamide (9a).—Ethyl cyano(1-ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylidene)acetate (**11**) (8.0 g) and 70% v/v of H_2SO_4 (80 ml) were heated at 70°, with stirring, for 90 min during which time CO_2 was evolved. The solution was poured onto ice and neutralized with NH_4OH and the precipitated product was recovered by filtration. Two recrystallizations from EtOH gave khaki needles, 3.46 g (54%). The compound slowly decomposes when heated above 180°.

The compounds listed in Tables II and III were prepared in a manner similar to that described above.

Biological Tests.—The methods used for determining the anti-inflammatory activity of the compounds were those previously reported by Cashin and Jackson,[1] and included the rat paw edema test, using carrageenin as the phlogistic agent, and the uv erythema test in guinea pigs.

Results and Discussion

The antiinflammatory results of the intermediate substituted cyanoacetates, the substituted acetamides, and the pyrido[3,4-*c*]quinoline-2,4-diones are recorded in Tables I–III.

Of the cyanoacetates, only ethyl cyano-4(1H)-quinolylideneacetate (**1**) showed any activity. Of the two simple acetamides prepared and tested, 4-quinolylacetamide (**1a**) showed fair activity in the carrageenin test, whereas 7-methyl-1,8-naphthyrid-4-ylacetamide (**3a**) showed no activity. Neither compound showed any activity in the uv erythema test.

The most active compound prepared, 1-ethyl-7-methyl-1,8-naphthyrid-4(1H)-ylideneacetamide (**9a**), showed fair activity in both tests. Replacement of the ethyl group by other alkyl or a substituted-alkyl group resulted in loss of activity. The same appears true of the pyrido[3,4-*c*]quinoline-2,4-diones where, although only three compounds have been prepared and tested, the ethyl derivative **7a** was the only derivative showing activity.

Acknowledgments.—Thanks are due to Mr. M. P. Fearnett for experimental assistance, to Dr. N. C. Franklin for spectroscopic data, and particularly to Mr. C. H. Cashin and Miss R. A. K. Finucane for biological tests and valuable discussion.

Retardation of Collagen Fibril Formation by Unsaturated Fatty Acids

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Received January 22, 1968

Long-chain unsaturated fatty acids retarded collagen fibrogenesis. When collagen was precipitated by warming neutral tropocollagen solutions to 37°, oleic, linoleic, palmitoleic, linolenic, and arachidonic acids at levels down to 1 μM increased the nucleation period and decreased the fibril growth rate. C_{12}–C_{22} saturated fatty acids, as well as dipalmitoyl lechithin, dipalmitoyl cephalin, and sodium lauryl sulfate, failed to alter the collagen precipitation.

The participation of collagen fibrosis at some stage in pathological and healing processes makes it essential to understand the nature of collagen precipitation from solution; a crucial aspect of this is the influence of pharmacological agents. This paper reports a significant influence on this phase transformation by long-chain unsaturated fatty acids.

Experimental Section

The unsaturated fatty acids included in this study, the position of their double bonds, and the number of their carbon atoms are palmitoleic, Δ^9-C_{16}; oleic, Δ^9-C_{18}; linoleic, $\Delta^{9,12}$-C_{18}; linolenic, $\Delta^{9,12,15}$-C_{18}; and arachidonic, $\Delta^{5,8,11,14}$-C_{20}. All of the unsaturated fatty acids except arachidonic acid were purchased from Applied Science Laboratories, Inc., State College, Pa., and were stated to be 99–100% pure. Arachidonic acid (90–95% purity), C_4–C_{22} saturated fatty acids (99–100% purity), and related compounds were purchased from the Hormel Institute, Austin, Minn.; tlc showed no oxidation contaminants in the arachidonic acid. Reduced DL-6,8-thioctic acid was purchased from Sigma Chemical Co. The unsaturated fatty acids were stored in the dark at 1° as 0.1 and 0.4% solutions in 95% EtOH.

At all but the lowest fatty acid concentrations, the presence of a solubilizing agent was necessary. For this purpose, we used EtOH at 10% except in several specified experiments in which the levels were lower. Qualitatively, the influence of EtOH alone varied with its concentration, as first observed by Bensusan;[1] with increasing EtOH concentration fibrogenesis was accelerated, then inhibited, and finally accelerated again. Therefore, collagen–ethanol controls were always run.

Collagen was isolated from the tail tendon of 6–8-week-old male Sprague–Dawley rats weighing 200–235 g. The isolation, extraction, and fibril precipitation were carried out as described previously.[2] Solutions were buffered in 0.13 *M* potassium phosphate, pH 7.4. Turbidity readings were made every minute for 10 min and every 2–5 min thereafter during a period of 20–40 min. As Wood[3] and other investigators have shown, the precipitation reaction comprises two stages: a lag period, during which nucleation occurs, and a fibril growth period. The over-all rate reflects both, and often long nucleation periods are accompanied by slow growth rates. The ultimate absorbance value, that value which no longer changes with time, is independent of both the lag time and the growth rate. It does, however, mark the time at which precipitation has become essentially complete.

The growth rate can be expressed either as the slope of the linear portion of the sigmoidal turbidity–time curve or by $T_{0.5}$, the half-growth time. The slope is a valuable indicator of the growth rate when at least one of the precipitations in a series

(1) H. B. Bensusan, *J. Am. Chem. Soc.*, **82**, 4995 (1960).
(2) N. H. Grant and H. E. Alburn, *Biochemistry*, **4**, 1271 (1965).
(3) G. C. Wood, *Intern. Rev. Connective Tissue Res.*, **2**, 1 (1964).