The oil was dissolved in 50% aqueous methanol and titrated with standard sodium hydroxide solution to *p*H 9.3 to convert small amounts of the hydrochloride of the starting monoquaternary chloride to monoquaternary chloride base, treated with carbon dioxide, evaporated down on the steam-bath *in vacuo* and recrystallized alternately from acetone–ethyl acetate and acetone–ether (twice from each), adding a small amount of water since the hydrate crystallized much more readily than the anhydrous bis-quaternary salt. Samples of the hydrate melted unreproducibly about 96–102°, so the progress to constant melting point was followed by drying samples at 80° (0.1 mm.). The anhydrous bis-quaternary so produced had a m.p. 176–177°. A total of 7.5 g. of γ-isomer of this m.p. was isolated. Analyses of both isomers will be found in the table.

1,4-2,5-*trans*-Tetramethyl-1,4-bis-dodecylpiperazinium Chloride, β- and γ-Isomers.—The 27.5 g. of the non-crystalline 1,4-2,5-*trans*-trimethyl-1,4-bis-dodecylpiperazinium chloride prepared by dodecylating the β-isomer of compound IV was treated with 25 g. of methyl iodide in 120 ml. of acetone. The solution was stored at 40° for 9 days. The solid (A) formed by this procedure (9.58 g.) was filtered, and the mother liquor (B) maintained at 40° an additional week. The solid A was converted to chloride by use of silver chloride in methanol, and recrystallized to give 5.43 g., m.p. 200.5–203°. This was combined with more solid melting in the same range which had been isolated by the chromatography procedure outlined below to give 7.56 g. of β-isomer of the bis-quaternary. This was recrystallized from nitromethane–ethyl acetate yielding 6.23 g., m.p. 202.5–205°.

The mother liquor B was evaporated down, leaving 28.1 g. of dark glass, which was converted to chloride with silver chloride in methanol to give 21 g. of oil. This was dissolved in boiling ethyl acetate after titrating with standard aqueous sodium hydroxide to *p*H 7 (it contained over 14 g. of monoquaternary starting material) and treated with acetone–ether to crystallize 660 mg. more of solid m.p. >200°. The residue of evaporation of the mother liquor was dissolved in benzene and chromatographed on an alumina column 77 cm. high and 6 cm. in diameter. Elution by means of 16 l. of benzene containing first 1.5% then 2.5% and finally 7.5% of ethanol gave small amounts of material, m.p. *ca.* 198–199°, then much uncrystallizable oil which was soluble in ether (presumably the starting bis-dodecyl monoquaternary). Most of this came out after elution with the first 1.5 l. of 7.5% ethanol in benzene. The next 500 ml. of this mixture gave an oil which, on extraction with ether, deposited crystals insoluble in the solvent. Elution of the column with absolute ethanol then gave 3.4 g. of residue insoluble in ether. Several recrystallizations from ethyl acetate gave 860 mg. of the bis-quaternary γ-isomer as hydrate, m.p. 97.5–101°. It contained no base or base hydrochloride detectable by the shape of its titration curve and, when dried, had m.p. 174–178.5°.

Anal. Calcd. for $C_{32}H_{68}N_2Cl_2$: N, 5.08. Found: N, 5.03.

TUCKAHOE 7, N. Y.

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

The Preparation and Stereochemistry of Some Cinnamonitriles, Some Cinnamamidines and Related Compounds

BY M. HARFENIST AND ARTHUR P. PHILLIPS

RECEIVED JULY 8, 1958

Mixtures of the *cis* and *trans* isomers of several substituted cinnamonitriles have been prepared by reaction of the benzaldehydes or aryl ketones with cyanoacetic acid followed by decarboxylation. Use of pyridine–piperidine as reaction medium led to the direct formation of a mixture containing about 75% of *trans*-nitrile in the case of *o*-chlorobenzaldehyde. A two-step procedure, comprising formation of apparently stereochemically homogeneous *o*-chlorobenzylidenecyanoacetic acid and its subsequent copper-catalyzed decarboxylation, gave a considerably greater proportion of *cis*-nitrile. The separation of these isomers of *o*-chlorocinnamonitrile and aspects of the infrared absorption curves of these and some related compounds are discussed. On treatment with various halomagnesium dialkylamides both *cis*- and *trans*-cinnamonitriles gave good yields of only the *trans*-cinnamamidines. N,N-Dibutyl-β-cyclocitrylidenoacetamidine (III) was made by a series of reactions in which the β-*trans* configuration of β-ionone was retained, as shown by comparison of the pertinent infrared and ultraviolet spectra with those of other substances of known configuration.

Several amidines have been reported to have local anesthetic activity.[1] Certain of these amidines, and in particular the cinnamamidines appear to possess antifibrillatory activity in the dog.[2] It therefore seemed to be worthwhile to prepare additional variants of the cinnamamidines. This paper reports work along these lines, as well as the preparation of an analogous amidine in which an alicyclic group replaced the phenyl group, and some incidental studies of the stereochemistry of the preparative methods for the cinnamonitriles.

In order to prepare the amidines of interest it was desirable to have a good general synthesis of the cinnamonitriles required as starting materials. We have used two methods of synthesis of the nitriles, both involving the condensation of aromatic aldehydes with cyanoacetic acid: (1) A modification of the Doebner synthesis[3] of cinnamic acids which involves heating the reactants in pyridine with piperidine as a catalyst. In this process, condensation is followed immediately by decarboxylation and the cinnamonitrile is isolated directly from the reaction mixture. (2) A two-step process consisting of: (a) base-catalyzed condensation of the reactants to give the benzylidenecyanoacetic acid, which is isolated; (b) decarboxylation, catalyzed by copper powder to give the cinnamonitrile. The former "Doebner-like" method generally was preferred, since it appeared to be quicker and more convenient.

Preparation of *p*-methylcinnamonitrile by this method gave a liquid cinnamonitrile, which was assumed to be a mixture of the *cis* and *trans* isomers. Since what is presumably the *trans p*-methylcinnamonitrile has been reported to melt at 79–80°,[4] attempts were made to separate this isomer by several redistillations, followed by crystallization, but this procedure was not successful. It would seem likely by comparison with the results reported with cinnamonitrile[5] and with our results (below) with *o*-chlorocinnamonitrile that this mixture would be more than half *trans*. However, in view of the

(1) E. Lorz and R. Baltzly, THIS JOURNAL, **70**, 1904 (1948); **73**, 483 (1951).

(2) Private communication from Dr. C. H. Ellis of these laboratories.

(3) J. R. Johnson, "Organic Reactions," Vol. I, R. Adams, Editor, John Wiley and Sons, Inc., New York, N. Y., 1942, pp. 222, 235 *et seq.*

(4) E. Fiquet, *Ann. chim. phys.*, [6] **29**, 479 (1893).

(5) E. J. Corey and G. Fraenkel, THIS JOURNAL, **75**, 1168 (1953).

results reported below, it was felt to be unnecessary for our purposes to isolate the pure *trans* isomer and so further attempts to do this were not carried out with this compound. Similarly, use of benzaldehyde, *o*-methylbenzaldehyde and *o*-chlorobenzaldehyde gave, by either of the cinnamonitrile methods above, mixtures of the corresponding two stereoisomeric cinnamonitriles.

It was of interest to determine the stereochemistry of the amidines produced by treatment of the mixed cinnamonitriles with halomagnesium dialkylamides, since these appeared to be single pure amidines rather than mixtures. In the event that they proved to be *trans*, as was anticipated and is shown below, it was felt desirable to know whether the *cis*-nitriles would give *cis*-amidines. To study this last point either pure *cis*-nitriles or *cis*-rich nitrile mixtures obviously would be required. It was therefore of interest to determine whether the copper-catalyzed decarboxylation would give more *cis*-nitrile than the relatively small amount expected at equilibrium, since Corey already has reported that a pyridine-catalyzed decarboxylation procedure led to essentially the equilibrium proportions of the isomers of cinnamonitrile.

Mixed *cis*- and *trans*-*o*-chlorocinnamonitriles were prepared from (apparently homogeneous) *o*-chlorobenzalcyanoacetic acid by copper-catalyzed decarboxylation. A *cis*-rich fraction was separated from the mixture by fractional distillation *in vacuo*, and was further purified to constant melting point by partial melting procedures, and finally recrystallized. It had little *trans* isomer, as indicated by infrared absorption analysis. Pure *trans*-nitrile was separated by flash distillation of the residue from the above distillation, with subsequent recrystallization to constant melting point.[6] This latter higher boiling and higher melting isomer was identified as *trans*-nitrile by its possession of a strong absorption band at 10.4 μ in the infrared, which was absent from the absorption of the *cis* isomer.[7]

Since pure *trans*-*o*-chlorocinnamonitrile and the essentially pure *cis* isomer were now available, it was possible to use their absorptions at 10.4 and at 12.93 μ, respectively, to determine the proportions of these isomers in the crude product of each of the preparative procedures. A single run of the "Doebner-like" nitrile preparation[8] indicated that 78% of the nitrile produced was the *trans* isomer. For comparison, essentially the same proportion, 74%, of the nitrile mixture produced by decarboxylation of *o*-chlorobenzylidenecyanoacetic acid in pyridine–piperidine at 155–160° was found to be the *trans* isomer. This *trans*/*cis* ratio presumably represents the equilibrium relationship of these isomers at about 150°. It is not unreasonable, in view of the greater bulk of the *o*-chlorophenyl group as compared to the phenyl group, that more *trans* isomer would be present at equilibrium in *o*-chlorocinnamonitrile than the 62 *trans*:38 *cis* relationship found[9] at equilibrium with cinnamonitrile.

In contrast to the pyridine–piperidine-catalyzed decarboxylation, the copper-catalyzed decarboxylation of *o*-chlorobenzylidenecyanoacetic acid gave slightly more *cis* than *trans* isomer, 52% of the total nitrile having been *cis* isomer. The cyanoacetic acid which had been decarboxylated appeared to be a single pure compound, hence stereochemically homogeneous. This presumably more stable cyanoacid would be expected to have its carboxyl group, which would be bulkier than the nitrile group, *trans* to the aryl group. Production of a proportion of *cis*-nitrile on decarboxylation far greater than that present at equilibrium would seem to confirm this assignment. If the production of *trans* isomer represents partial equilibration of nitrile after its formation but during its brief residence at the high decarboxylation temperature, the rate of isomerization of the *cis*-*o*-chlorocinnamonitrile must be greater than that found for cinnamonitrile[9] by a factor of 10^5 or more. Alternatively, isomerization may occur during the decarboxylation.[10]

In addition, cinnamonitrile was partially separated into its *cis*–*trans* isomers by a procedure analogous to that of Ghosez,[11] after its preparation by the "Doebner" procedure.

The pure *trans* isomer of *o*-chlorocinnamonitrile, m.p. 41–42°, was converted by the action of bromomagnesium dibutylamide to an amidine (I) in 82% yield, recrystallized to constant melting point. This had a strong absorption band at 10.25 μ,[12] and so was the expected *trans*-amidine.

(6) The *trans*-nitrile has been prepared by dehydration of the *trans*-amide [G. Lasch, *Monatsh.*, **34**, 1653 (1913)] but it was thought to be advisable to confirm the assignment of *trans*-configuration to the product.

(7) All nitrile absorptions were determined in carbon disulfide solution. Corresponding to observations made in the polyene field [W. Oroshnik and A. D. Mebane, THIS JOURNAL, **76**, 5719 (1954)] the *cis*-nitrile could not be unequivocally identified from its infrared absorption except by comparison with the *trans*-nitrile. However, our relatively homogeneous *cis*-*o*-chlorocinnamonitrile and our *cis*-rich nitrile fractions in the other cases all showed strong absorption maxima at 12.9 ± 0.1 μ, which were absent from the *trans*-nitrile absorptions. This maximum presumably represents the *cis* peak, usually reported at about 13.5–13.5 μ, lowered in wave length by conjugation. A similarly low wave length absorption at about 12.8 μ, not found in the *trans* isomer, has been reported for *cis*-β-carotene [J. D. Surmatis, J. Maricq and A. Ofuer, *J. Org. Chem.*, **23**, 157 (1958)]. Similar data and references are in J. L. H. Allan, G. D. Meakins and M. C. Whiting, *J. Chem. Soc.*, 1874 (1955). All of the compounds reported here showed various absorption peaks above 13 μ, as would be anticipated for substituted aromatic compounds, and all of the *trans*-amidines had absorption maxima at about 10.25 μ (see below).

(8) The "Doebner-like" nitrile preparation, after three hours of heating at 135–140°, still gave over 14% of undecarboxylated *o*-chlorobenzalcyanoacetic acid. This temperature and time in our hands has been sufficient to allow essentially complete decarboxylation of unsubstituted benzalcyanoacetic acid, and so represents another example of steric hindrance to decarboxylation due to *ortho* groups (*cf.* ref. 5).

(9) G. B. Kistiakowsky and W. R. Smith, THIS JOURNAL, **58**, 2428 (1936).

(10) It seems reasonable that the decarboxylation proceeds *via* an intermediate such that the loss of carbon dioxide would lead to an anionic condition of the carbon bearing the nitrile group. Such an intermediate may have copper bonded to the nitrile nitrogen [*cf.* E. J. Corey, THIS JOURNAL, **75**, 1163 (1953)] but, whatever the finer details of its structure, would presumably be of low stereochemical stability. An alternative possibility is that the *trans*-nitrile is produced by a very rapid decarboxylation of the less stable benzylidenecyanoacetic acid isomer which has its nitrile group *trans* to the aryl group. This would be present in low concentration, although the greater acidity to be expected for a carboxyl group *cis* to the phenyl compared to one *trans* to phenyl (see the pK_a values for the cinnamic acids) might partially compensate for the concentration factor. In the absence of data on the rate of isomerization of the more stable to the less stable cyanoacetic acid isomer, it is impossible to exclude this mechanism.

(11) J. Ghosez, *Bull. soc. chim. Belg.*, **41**, 477 (1932).

(12) All amidine hydrochloride infrared absorptions were determined in KBr pellets.

$$\underset{\textit{cis or trans}}{ArCH{=}CH{-}CN} + N(C_4H_9)_2MgBr \longrightarrow$$

$$ArCH{=}CH{-}\overset{\overset{NH}{\|}}{C}{-}N(C_4H_9)_2(\cdot HCl)$$

trans

I

By the same procedure a *cis-o*-chlorocinnamonitrile fraction of m.p. 14–14.8° (our purest *cis* isomer melted 14.2–14.8°[13]) gave a 59% yield, recrystallized to constant melting point, of the same amidine hydrochloride as had the *trans*-nitrile. Results of the treatment of *cis*-rich fractions of cinnamonitrile with iodomagnesium piperidide, analogously, were the same as those obtained with *trans*-cinnamonitrile and the same reagent.

No attempt was made to determine whether isomerization had occurred before, during, or after amidine formation, nor is it possible to rule out the existence of a small portion of *cis*-amidine in the mother liquors of the *cis*-nitrile reaction. However, in view of the difficulties inherent in purification in this series of compounds, it is obvious that this reaction is impractical as a method of preparing *cis*-cinnamamidines, and it is apparent that only *trans*-amidines will be isolated even when mixed *cis*- and *trans*-cinnamonitriles are used as starting materials.

The properties found for the hydrochlorides of some other new *trans*-cinnamamidines prepared in the course of our investigations are given in Table II. In most cases these salts crystallized directly on addition of aqueous hydrochloric acid to the reaction mixture, despite the presence of bromide ion from the ethyl bromide or iodide from ethyl iodide used in the initial Grignard reaction. In several other cases a mixture of amidinium chloride and bromide or iodide was produced and the (usually yellow) solid had to be treated with silver chloride in methanol to convert all the amidine to hydrochloride. Yields of amidines in general averaged 60–85%.

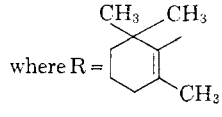
As an alicyclic analog of the cinnamamidines reported here, the amidine III, in which the 2,6,6-trimethylcyclohexenyl group replaces the aryl group of the cinnamamidines, was made. The nitrile required, II, reportedly has been made by cyclization of citrylideneacetonitrile,[14] under conditions which probably give a mixture of mostly α- and some β-nitrile. The route reported here is illustrated below. It starts with the known β-acid prepared[15] by hypochlorite oxidation of β-ionone.

$$RCH{=}CH{-}COOH \longrightarrow RCH{=}CH\overset{\overset{O}{\|}}{C}NH_2 \longrightarrow$$

$$RCH{=}CH{-}CN \longrightarrow RCH{=}CH\overset{\overset{NH}{\|}}{C}N(C_4H_9)_2{\cdot}HCl$$

II III

where R =



Thionyl chloride gave the acid chloride which was not purified but, after removal of excess thionyl chloride, was treated with dry ammonia in ether to give a nearly quantitative yield of crystalline amide whose melting point was raised only slightly on recrystallization. Dehydration with thionyl chloride in refluxing benzene[16] converted the amide to a liquid nitrile which, on treatment with bromomagnesium dibutylamide, gave an excellent yield of the dibutylamidine hydrochloride III, whose melting point was not raised on recrystallization. The excellent yields and the high purity of the crystalline compounds suggest that the reaction sequence used leads to the β-*trans*-amidine without rearrangement of either double bond. Further indication of this is available from a comparison of the pertinent ultraviolet absorption data (Table I).

TABLE I

ULTRAVIOLET ABSORPTION DATA FOR AMIDINE HYDROCHLORIDES AND FOR RELATED KETONES IN 95% ETHANOL

	λ_{max}, mμ	ϵ_{molar}
N,N-Dibutyl-β-cyclocitrylidene-acetamidine (III)	289.5	8,800
β-Ionone[a]	295	10,700
N,N-Di-*n*-amylcinnamamidine	284.5	21,600
Benzalacetone[b]	285	23,000

[a] W. G. Young, S. J. Cristol, L. J. Andrews and S. L. Lindenbaum, THIS JOURNAL, 66, 855 (1944). [b] T. M. Lowry, H. Moureu and C. A. H. MacConkey, *J. Chem. Soc.*, 3167 (1928).

It will be seen that the position and intensity of absorption of each amidine is in good agreement with the published values for the corresponding ketone. The *trans* configuration of the diamylcinnamamidine is known unequivocally from the existence in its infrared spectrum of an absorption band at 10.22 μ, as well as by analogy with the N,N-dibutyl-*o*-chlorocinnamamidine discussed earlier. The usual form of benzalacetone is *trans*[17] as are the substituents about the double bond which is in the side-chain of β-ionone.[18]

The *trans* configuration of the side-chain double bond substituents of the cyclocitrylideneamidine III is further shown by its *trans* absorption maximum at 10.30 μ.

Acknowledgments and Instrumentation.—The infrared absorption data and analyses were determined on Beckman IR2A (nitriles) and IR4 (amidines) spectrophotometers by Mr. James Murphy and Mr. Patrick Tolve, of our Analytical Research Laboratories, the ultraviolet data on a Beckman DK 2 instrument by Rosario Orsini of our Chemotherapy Division. Nitrogen analyses were done by the Kjeldahl method by Veronica Purdy. Carbon–hydrogen analyses were performed by Mr. S. Blackman and Mr. C. K. Marr.

(13) Estimated 6% *trans-o*-chlorocinnamonitrile by assuming that the absorption of pure *cis* at 10.4 μ can be obtained by drawing a line connecting the two flanking absorption minima (at 10.0 and 10.45 μ) and reading the intercept of this line at 10.4 μ.

(14) A. Verley, German Patent 153,575; *Chem. Centr.*, **75**, II, 677 (1904).

(15) W. G. Young, L. G. Andrews and S. J. Cristol, THIS JOURNAL, **66**, 520 (1944).

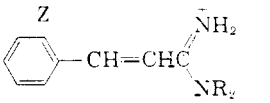
(16) A. Michaelis and H. Siebert, *Ann.*, **274**, 312 (1893).

(17) G. van Bree, *Bull. soc. chim. Belg.*, **57**, 71 (1948); E. Baroni and H. Seibert, *Naturwiss.*, **29**, 560 (1941).

(18) *Cf.* W. Oroshnik, G. Karmas and R. A. Mallory, THIS JOURNAL, **76**, 2325 (1954).

TABLE II

trans-CINNAMAMIDINIUM SALTS



	Z	NR_2	X^-	M.p., °C.[a]	Recrstn. solvents[b]	Formula	Analyses, % Calculated C	Calculated H	Found C	Found H
1	H	$(C_2H_5)_2N$	Cl	182–190	A–Æ	$C_{13}H_{19}ClN_2$	N, 11.7		N, 11.9	
2	H	$(n\text{-}C_3H_7)_2N$	Cl	227–228	A–Æ	$C_{15}H_{23}ClN_2$	67.6	8.7	67.5	8.4
3	H	$(C_4H_9)_2N$[c]	Cl	211–212	N–E	$C_{17}H_{27}ClN_2$	Cl^-, 12.0		Cl^-, 12.2	
4	H	$(n\text{-}C_5H_{11})_2N$	Cl	206–208	A–Æ–H	$C_{19}H_{31}ClN_2$	70.8	9.7	70.7	9.6
5	H	$(n\text{-}C_6H_{13})_2N$	Cl	184–185	A–E	$C_{21}H_{35}ClN_2$	N, 8.0		N, 7.7	
6	H	$(CH_2)_4N$	Br	252–253	A	$C_{13}H_{17}BrN_2$	55.5	6.1	55.8	5.8
7	H	$(CH_2)_5N$	Cl	240–241	A–E	$C_{14}H_{19}ClN_2$	67.0	7.6	67.0	7.4
8	H	$C_7H_{14}N$[d]	Cl	243–244	A	$C_{16}H_{23}ClN_2$	68.9	8.3	69.1	8.2
9	CH_3	$(n\text{-}C_4H_9)_2N$	Cl	208	A–Æ–E	$C_{18}H_{29}ClN_2$	Cl^-, 11.5		Cl^-, 11.3	
10	Cl	$(n\text{-}C_4H_9)_2N$[e]	Cl	225–229	A–Æ	$C_{17}H_{26}Cl_2N_2$	Cl^-, 10.8		Cl^-, 11.0	
11	H	$(n\text{-}C_4H_9)_2N$[e,f]	Cl	148.5–150.3	Æ–E–P	$C_{18}H_{29}ClN_2$	N, 9.1		N, 9.2	
12	H	$(CH_2)_5N$	..[g]	106–107	H	$C_{20}H_{22}N_2$	82.7	7.6	82.7	7.4

[a] M.p.'s not corrected. [b] Solvents: A = absolute ethanol, Æ = ethyl acetate, E = anhydrous ether, H = hexane, N = nitromethane, P = pentane. [c] C_4H_9 = isobutyl. [d] *cis*-2,6-Dimethylpiperidino. [e] Details of this preparation are given in the experimental section. [f] This compound has a β-methyl substituent. [g] This compound has an α-phenyl substituent. Analysis and m.p. are of the base. The hydrochloride had m.p. 240–241° after recrystn. from A–E.

Experimental[19]

Cyanoacetic Acids.—The procedure of reference 20 was modified by using 1.15 moles of commercial cyanoacetic acid, previously treated with an equivalent amount of sodium carbonate, per mole of aldehyde. Yields of over 90% of the benzalcyanoacetic acids were obtained by this procedure from benzaldehyde, *o*-methylbenzaldehyde and *o*-chlorobenzaldehyde, but it failed with β-cyclocitral.

Cinnamonitriles.—The decarboxylation of the cyanoacetic acids was accomplished by heating them with copper powder, essentially as outlined below for *o*-chlorocinnamonitrile. No other nitrile, however, was separated into the isomers.

2-Methylcinnamonitrile was prepared in 70% yield from the cyanoacetic acid by this procedure, and had b.p. 147–150° (24 mm.).

Anal. Calcd. for $C_{10}H_9N$: C, 83.93; H, 6.34. Found: C, 84.02; H, 6.34.

***cis*- and *trans*-*o*-Chlorocinnamonitriles by Copper-catalyzed Decarboxylation**—The crude first crop of *o*-chlorobenzalcyanoacetic acid from 170 g. of *o*-chlorobenzaldehyde was heated with 3 g. of copper powder under water-pump vacuum at a temperature (bath temp. 270°) which caused vigorous evolution of carbon dioxide. The distillate, 128 g., was redistilled over copper powder collecting the fraction b.p. 129–134° (8 mm.), m.p. *ca.* 3°. (The product of a subsequent run was found to be approximately 52% *cis*- and 48% *trans*-nitrile.) The last portion of this distillate solidified in the condenser. A 54-g. portion of the total distillate was therefore distilled through a concentric tube column, taking 28.2 g., b.p. 88–93° (0.09 mm.), m.p. 8–10°. It was purified by cooling it in cold water with stirring, filtering off the solid when about half had solidified, and melting and half-freezing the solid three times more, centrifuging and decanting the liquid each time. The solid residue then had m.p. 14.3–14.8°, not raised on recrystallization from 1:6 anhydrous ether–petroleum ether by cooling to −15°. Further work-up of the mother liquors by fractional crystallization from the same solvent mixture gave a total of 9.76 g., m.p. >14°, depressed when admixed with the *trans*-nitrile.

Pure *trans*-nitrile was obtained by flash distilling the residue left after distillation of the *cis*-isomer through the column. A total of 19.5 g., b.p. 104° (0.8 mm.), was obtained, from which 14.4 g. of m.p. >40° was obtained by recrystallization from 50% anhydrous ether–petroleum ether. The best fraction had m.p. 41–42°. A melting point of 40° has been reported[2] for presumably *trans*-nitrile.

***o*-Chlorocinnamonitrile by the Pyridine–Piperidine Method.**—A mixture containing 150 cc. of pyridine, 140 g. (112 cc., 1 mole) of *o*-chlorobenzaldehyde, 90 g. (1.05 moles) of cyanoacetic acid and 10 cc. of piperidine was heated for two hours at 100° (steam-bath) and then was refluxed in a metal-bath at 135–140° for three hours longer. After having been cooled, the reaction mixture was dissolved in 2 liters of ether and was washed with two 200-cc. portions of cold water, then with four 150-cc. portions of 4 *N* hydrochloric acid (the last 2 portions gave *p*H 1). The aqueous and acid washes were combined and gave a heavy white crystalline precipitate (A) which was shown to be the intermediate *o*-chlorobenzylidenecyanoacetic acid. The ether layer was next washed with two 150-cc. portions of 10% aqueous potassium carbonate solution. A white precipitate (B) formed immediately upon addition of the carbonate solution. This precipitate was filtered off and when treated with aqueous hydrochloric acid gave more of the acid (A). The aqueous carbonate filtrates were used to re-wash the ether layer and then were discarded.

The acid fraction A (from both A and B above) amounted to about 44 g. and after several recrystallizations from methanol this gave 28 g. (14% based on starting aldehyde) of the pure *o*-chlorobenzylidenecyanoacetic acid, m.p. 216–217°.

Anal. Calcd. for $C_{10}H_6ClNO_2$: C, 57.9; H, 2.9. Found: C, 58.2; H, 2.9.

The washed ether layer was dried over anhydrous potassium carbonate and after filtration and evaporation gave 132 g. (80% yield if this were all the chlorocinnamonitrile). Distillation in *vacuo* gave 115 g. of material collected in four arbitrarily separated fractions boiling between 90° and 220° at 13 mm. After several redistillations these fractions were redistributed: I, 17 g., b.p. 90–133° at 13 mm.; II, 12 g., b.p. 143–148° at 13 mm.; III, 29 g., b.p. 147–151° at 13 mm.; IV, 31 g., b.p. 153–156° at 13 mm.; V, 16 g., b.p. 185–210° at 13 mm.

Fraction I was principally recovered *o*-chlorobenzaldehyde and was characterized and identified as its semicarbazone, which had the same m.p. as a sample prepared from known

(19) Analytical data given in Table II are not repeated in the preparative detail in this section.

(20) "Organic Syntheses," Coll. Vol. I, second edition, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 181.

o-chlorobenzaldehyde, *ca.* 243–245°. Based on the yield of semicarbazone obtained fraction I must have been >80% aldehyde—this would represent a 10–15% recovery of the starting aldehyde.

Fractions II, III and IV represent the cinnamonitrile yield. Basing the *cis–trans* composition of each fraction on infrared analyses and using the spectra of the pure isomers as reference, the composition of the three fractions was: II, 51% *trans* and 42% *cis;* III, 64% *trans* and 25% *cis;* IV, 83% *trans* and 7% *cis*.

The total combined yield of II, III and IV was 72 g. (45%) of the cinnamonitrile and this consisted of 56 g. (78%) *trans* and 16 g. (22%) *cis*.

Fraction V, 16 g., probably consisted largely of β-(*o*-chlorophenyl)-glutaronitrile and was not further investigated.

Amidines.—All were made essentially by the procedure of Lorz and Baltzly[1] using dried benzene to increase the solubility of the reactants where necessary. Either the hydrochloride or a mixture of hydrochloride with (usually yellow) hydrobromide crystallized during the decomposition. A typical example is given below.

N,N-Dibutyl-*o*-chlorocinnamamidine Hydrochloride.—To a Grignard reagent made from 3.09 g. (0.127 mole) of magnesium turnings, 14.2 g. (0.13 mole) of ethyl bromide and 120 ml. of absolute ether was added during 5 minutes 17.05 g. (0.132 mole) of di-*n*-butylamine dissolved in 50 ml. of anhydrous ether. The mixture was boiled for an additional half-hour, and a solution of 14.5 g. (0.089 mole) of *trans-o*-chlorocinnamonitrile of m.p. >40°, dissolved in 50 ml. of absolute ether, was added in a few minutes. The solution was boiled under reflux for 5 hours, and decomposed, after remaining overnight, by the addition of 50 ml. of 4 *N* HCl. The resulting solid was filtered, washed with 4 *N* HCl and then with ether. The residue was 27.7 g. of product, m.p. 210–222°. This was recrystallized from absolute ethanol–ethyl acetate giving a first crop of 15.6 g., m.p. 225–229°. A second crop obtained by addition of ether weighed 8.2 g., m.p. 227–228°.

N,N-Di-*n*-butyl-β-methylcinnamamidine hydrochloride was prepared from 59.7 g. of β-methylcinnamonitrile and bromomagnesium dibutylamide, essentially by the procedure given above. Decomposition of the reaction mixture with aqueous hydrochloric acid led to the formation of three layers. The middle one, which was benzene soluble, and the aqueous layer, were treated with an excess of iced aqueous sodium hydroxide and the amidine base so liberated was taken up in ether, dried briefly over magnesium sulfate and distilled. A fore-run of solvent and of dibutylamine (the latter removed *in vacuo*) was followed by 10.07 g. (12% of theory) of distillate, b.p. 120–126° (0.15 mm.). This was acidified in ethyl acetate–ether solution with gaseous hydrogen chloride, and treated with Skellysolve A to give an oil. This was redissolved and, after a week at −15°, a crystalline solid was formed. This was recrystallized from the same solvent mixture, m.p. 148.5–150.3°.

β-Cyclocitrylideneacetamide.—To 15.7 g. (0.081 mole) of cyclocitrylideneacetic acid was added 48 g. of thionyl chloride. After the acid had dissolved and the mixture had been heated for 40 minutes under reflux, the excess thionyl chloride was evaporated at the water-pump up to bath temperature 42°. The residual oil was taken up in 80 ml. of absolute ether and added dropwise to 200 ml. of absolute ether kept saturated with ammonia by passing in a rapid stream of gaseous ammonia throughout the addition and for 10 minutes more. The solution then was filtered to remove ammonium chloride, and the ethereal solution was washed with water, dried and concentrated. The residue was 15.4 g., m.p. 143–146°. This was recrystallized from Skellysolve C to give 13 g. of white solid, m.p. 147–148°.

Anal. Calcd. for $C_{12}H_{19}NO$: N, 7.25. Found: N, 7.54.

β-Cyclocitrylideneacetonitrile (II).—A mixture of 11.4 g. (0.059 mole) of β-cyclocitrylideneacetamide and 70 ml. of benzene (dried by azeotropic distillation) was heated under reflux while 7.1 g. (0.065 mole) of thionyl chloride was added dropwise over 10 minutes. An additional 20 ml. of benzene was added and the solution was boiled an additional 2 hours. It was then distilled *in vacuo* collecting 9.94 g. (96% of theory, if pure) of a yellow liquid, b.p. 132–136° (12 mm.). This was redistilled at the same temperature [literature[5] b.p. 141° (17 mm.)].

N,N-Dibutyl-β-cyclocitrylideneacetamidine Hydrochloride.—The procedure previously given for cinnamamidines was followed with 9.94 g. of β-cyclocitrylideneacetonitrile except that the reaction mixture was decomposed after 2.5 hours under reflux, by addition of aqueous acid. The precipitate was filtered off, and washed with water and ether to give 17.7 g. of solid, m.p. 195–200°. This was dissolved in 500 ml. of boiling acetone, filtered from some inorganic impurity, and absolute ether was added to incipient turbidity (*ca.* 800 ml.). The first crop of felted needles had m.p. 192–198°, and had a broad absorption maximum at 289–290 mμ, ε 8800.

Anal. Calcd. for $C_{20}H_{37}Cl^-N_2$: Cl^-, 10.42. Found: Cl^-, 10.65.

TUCKAHOE, N. Y.

[CONTRIBUTION FROM THE KETTERING–MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE]

Synthetis of Potential Anticancer Agents. XVI. S-Substituted Derivatives of 6-Mercaptopurine[1a]

BY THOMAS P. JOHNSTON, LEE B. HOLUM AND JOHN A. MONTGOMERY

RECEIVED JUNE 30, 1958

A number of S-substituted derivatives of 6-mercaptopurine have been prepared by a new procedure in which dimethylformamide was used as the reaction medium.

Because both 6-methylthiopurine and 6-benzylthiopurine have shown activity against Adenocarcinoma 755[2] and Sarcoma 180[3] comparable to that of 6-mercaptopurine, an extensive investigation of the anticancer activity of S-substituted derivatives of 6-mercaptopurine is of great interest. For this study we have prepared the varied series of 6-alkyl- and 6-arylthiopurines summarized in Tables I and II. Although the screening of these compounds has not yet reached the stage at which a pattern of the structural effect on anticancer activity can be established, some interesting variations in activity and toxicity already have been noted. Speculation that the activity of the methyl and benzyl derivatives is related to an *in vivo* formation of 6-mercaptopurine by a biological cleavage of the thioether has been advanced.[4] Such a mechanism is

(1) Affiliated with Sloan–Kettering Institute. This work was supported by funds from the C. F. Kettering Foundation and the National Institutes of Health, Contract No. SA-43-ph-1740. Part XV. H. J. Schaeffer and H. J. Thomas, THIS JOURNAL, **80**, 4896 (1958).

(1a) Name in common usage; 6-purinethiol is used by "Chemical Abstracts."

(2) H. E. Skipper, J. A. Montgomery, J. R. Thomson and F. M. Schabel, Jr., *Proc. Am. Assoc. Cancer Research*, **2**, 346 (1958).

(3) D. A. Clarke, G. B. Elion, G. H. Hitchings and C. C. Stock, *Cancer Research*, **18**, 445 (1958).

(4) G. B. Elion, G. H. Hitchings, D. A. Clarke and C. C. Stock, *Proc. Am. Assoc. Cancer Research*, **2**, 199 (1957).