methylformamide–water gave an off-white solid, m.p. 228.5–230.5° dec. (resolidifies at *ca.* 232° and remelts at *ca.* 286.5–289°).

*Anal.* Found: C, 54.62; H, 4.29; N, 16.89.

This product was shown to be identical with the original sample by mixture melting point and infrared spectrum, and by spectrophotometric and paper chromatographic comparisons. Compound V gave a negative Bratton–Marshall test.

**N-(2-Methoxy-4-quinazolyl)benzenesulfonamide** was prepared from I and sodium benzenesulfonamide in 27% yield by the procedure given for III; m.p. 230–232°.

*Anal.* Calcd. for $C_{15}H_{13}N_3O_3S$: N, 13.30; S, 10.17. Found: N, 13.11; S, 10.25.

When a similar reaction was run with sodium N-methylbenzenesulfonamide and I, no product was isolated. The only crystalline solid isolated was 2-methoxyquinazolin-4(3H)-one.

**Acknowledgment.**—We wish to thank the following who gave their assistance: Messrs. D. H. Causey, W. M. Coates, J. P. Elkins, W. B. Lacefield, D. R. Stone, D. L. Wedding, E. F. Harrison, C. W. Stott, H. C. Hawkins, and E. H. Lash.

## Reactions between Orthoesters and Organic Nitrogen Compounds. VI.[1] Arylhydrazines

C. RUNTI AND C. NISI

*Pharmaceutical Chemistry Department, University of Trieste, Trieste, Italy*

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The reaction between ethyl orthoformate (EOF) and hydrazines has not yet been thoroughly investigated.[2–4] It is known that hydrazine hydrate itself gives 4-amino-4H-1,2,4-triazole[2] and that the reaction between EOF and phenylhydrazine, in the presence of acetic acid, produces N-formylhydrazine and 1,5-diphenylformazan.[3,4] We have therefore studied systematically the reactivity of the arylhydrazines with EOF and other orthoesters.

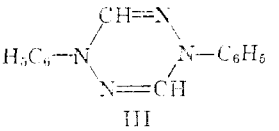
2-Nitro-, 4-nitro-, and 2,4-dinitrophenylhydrazine and their hydrochlorides react with EOF and other orthoesters to produce arylhydrazones of the ethyl esters of the corresponding carboxylic acids (I)[5] (Table I). In some cases, with EOF, the corresponding hydrazidines, *i.e.*, the N,N′-bis(arylamino)formamidines (II), may also be formed as by-products.

The presence of the ethoxy group in I is proved by their reactivity with aniline and with arylhydrazines. In this case compounds II are produced again.


I II

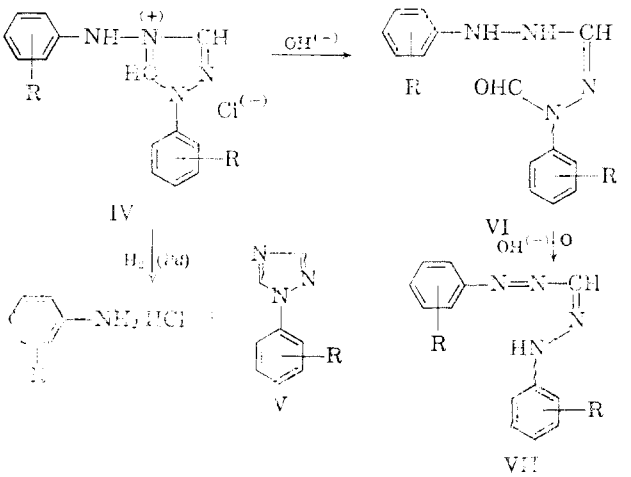
Phenylhydrazine and the tolylhydrazine hydrochlorides present, however, a completely different behavior. Two products were isolated by reaction between excess EOF and phenylhydrazine hydrochloride: the first (m.p. 198–198.5°) corresponds to the already known 1,4-dihydro-1,4-diphenyl-1,2,4,5-tetrazine (III); the second, obtained as the main product, melts at 210° dec. and has the molecular formula $C_{14}H_{13}ClN_4$. It is slightly soluble in water, contains ionic chloride, and yields a nitrate (m.p. 167.5–168°) which is hardly soluble in water. It is impossible to isolate the corresponding base, even under mild reaction conditions ($Ag_2O$).

For the second product the following considerations supported the structure 4-anilino-1-phenyl-1H-1,2,4-triazolium (4) chloride (IV, R = H). (a) By hydrogenolysis with palladium on carbon in ethanol, aniline hydrochloride and 1-phenyl-1H-1,2,4-triazole (V, R = H) are obtained. (b) By the action of aqueous ammonia on IV (R = H) a hydrolysis product, $C_{14}H_{14}N_4O$, m.p. 163.5–164° dec., is obtained. Elementary analysis and the presence of an absorption band at 1676 cm.$^{-1}$ in the infrared spectrum led us to assign the structure of N-(anilino)-N′-(formylanilino)formamidine (VI, R = H) to the compound. Further evidence was given by oxidizing VI (R = H) with hydrogen peroxide in alkaline medium, whereby 1,5-diphenylformazan (VII, R = H) was obtained.




III

Quite similar results were obtained starting from *p*-tolylhydrazine hydrochloride and EOF. Even in this case the structure was confirmed by a series of similar reactions. Compounds IV so obtained are




listed in Table II.

The structure of compounds IV is rather unexpected, since they are triazolium derivatives in which a quaternary nitrogen is bound to an arylamino group. They present in the infrared spectrum a rather broad absorption band at 1810 cm.$^{-1}$, attributable to the structural element $\geq N^+$-H (immonium band).[6] The compounds possess surface-active properties (concentration 4 mg./100 ml. at 25°), even though much lower than an equiconcentrated solution of dodecyl-*p*-tolyltrimethylammonium methylsulfate.[7]

(1) Paper V of this series: C. Ronti, C. Nisi, and L. Sindellari, *Ann. Chim.*, **51**, 719 (1961).

(2) R. Stollé, *J. prakt. Chem.*, [2] **68**, 467 (1903); see also C. F. Allen and A. Bell, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 96.

(3) L. Claisen, *Ann.*, **287**, 360 (1895).

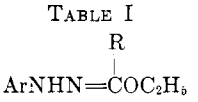
(4) R. v. Walther, *J. prakt. Chem.*, [2] **53**, 475 (1896).

(5) Only two examples of compounds of this type, obtained by E. Schmidt [*Ber.*, **47**, 2545 (1914)] by reaction between phenylhydrazine hydrochloride and an iodino ether, are mentioned in literature, to our knowledge. Generally iodino ethers and arylhydrazines react to give formazans and/or amidrazones [see A. W. Nineham, *Chem. Rev.*, **55**, 355 (1955)].

(6) B. Witkop, J. B. Patrick, and H. M. Kissman, *Chem. Ber.*, **85**, 949 (1952); see J. T. Potts, *Chem. Rev.*, **61**, 87 (1961).

(7) Desogen®.

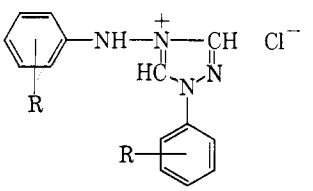
TABLE I



| I[a] | Ar | R | M.p., °C. | Yield, % | Formula | Calcd., % C | Calcd., % H | Calcd., % N | Found, % C | Found, % H | Found, % N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| a | 4-$O_2NC_6H_4$– | H | 147 | 45 | $C_9H_{11}N_3O_3$ | 51.67 | 5.30 | 20.09 | 51.57 | 5.49 | 20.41 |
| b | 4-$O_2NC_6H_4$– | $CH_3$ | 140.5 | 86 | $C_{10}H_{13}N_3O_3$ | 53.80 | 5.87 | 18.83 | 53.53 | 5.74 | 18.90 |
| c | 2-$O_2NC_6H_4$– | H | 51 | 86 | $C_9H_{11}N_3O_3$ | 51.67 | 5.30 | 20.09 | 52.11 | 5.44 | 20.05 |
| d | 2-$O_2NC_6H_4$– | $CH_3$ | 78.5 | 75 | $C_{10}H_{13}N_3O_3$ | 53.80 | 5.87 | 18.83 | 53.95 | 5.87 | 18.83 |
| e | 2-$O_2NC_6H_4$– | $C_6H_5$ | 99 | 52 | $C_{15}H_{15}N_3O_3$ | 63.14 | 5.30 | 14.73 | 63.13 | 5.17 | 14.58 |
| f | 2,4-$(O_2N)_2C_6H_3$– | H | 125.5 | 55 | $C_9H_{10}N_4O_5$ | 42.53 | 3.97 | 22.04 | 42.96 | 3.91 | 22.21 |
| g | 2,4-$(O_2N)_2C_6H_3$– | $C_6H_5$ | 156.5 | 31 | $C_{15}H_{14}N_4O_5$ | 54.54 | 4.27 | 16.96 | 54.39 | 4.19 | 17.18 |

[a] All the compounds listed were recrystallized from ethanol except Ia which was recrystallized from benzene.

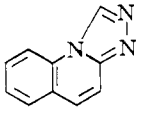
TABLE II



| IV[a] | R | M.p., °C.[b] | Yield, % | Formula | Calcd., % C | Calcd., % H | Calcd., % Cl | Calcd., % N | Found, % C | Found, % H | Found, % Cl | Found, % N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | $C_6H_5$ | 210 | 61 | $C_{14}H_{13}ClN_4$ | 61.65 | 4.80 | 13.00 | 20.54 | 61.98 | 4.99 | 13.00 | 20.21 |
| b | *p*-$CH_3C_6H_4$ | 187.5 | 46 | $C_{16}H_{17}ClN_4$ | 63.89 | 5.69 | 11.79 | 18.63 | 63.82 | 5.83 | 11.66 | 18.28 |
| c | *m*-$CH_3C_6H_4$ | 198 | 85 | $C_{16}H_{17}ClN_4$ | 63.89 | 5.69 | 11.79 | 18.63 | 63.67 | 5.79 | ... | 18.75 |
| d | *o*-$CH_3C_6H_4$ | 168 | 28 | $C_{16}H_{17}ClN_4$ | 63.89 | 5.69 | 11.79 | 18.63 | 63.99 | 5.57 | ... | 18.60 |

[a] All the compounds listed were recrystallized from ethanol and ether. [b] All melting points are with decomposition.

Finally, by the reaction between 2-quinolylhydrazine and EOF, *s*-triazolo[4,3-*a*]quinoline (VIII) was obtained; this compound had been prepared by Marckwald and Meyer[8] from 2-quinolylhydrazine with formic acid.



VIII

**Screening Data.**—Compounds IVa–d lower the carotid blood pressure when injected (5–10 mg./kg. i.v.) into anesthetized rats. The hypotensive effect seems to be due to ganglionic blocking, since they prevent, to different degrees, the hypertensive action of ganglionic stimulants like dimethylphenylpiperazinium iodide. These compounds are devoid of antiepinephrine and atropine-like properties (up to 10 mg./kg. i.v.). KB human tumor cells in logarithmic growth have been used to test the cytotoxic activity of IVa–d.[9] The four compounds at 1 $\gamma$/ml. are very active growth inhibitors. Compounds V (R = H) (as hydrochloride), VI (R = H), and VII (R = H) are inactive; apparently, the cytotoxic activity seems to be connected with the presence of the arylamino–triazolium structure.[10] The compounds IVa and b do not prevent growth of *Mycobacterium pyogenes var. aureus*, *Mycobacterium flavus*, *Escherichia coli*, and *Klebsiella pneumoniae* (tryptose broth, 24 hr. at 37°) at 1000 $\gamma$/ml. Preliminary toxicity data for compounds IVa–d give the following $LD_{50}$ (mg./kg., in mice, i.p.): IVa, 125; IVb, 150; IVc, 115; IVd, 90.

(8) W. Marckwald and E. Meyer, *Ber.*, **33**, 1885 (1900).

(9) L. Morasca, *Rev. Franc. études clin. biol.*, in press.

(10) The antitumor activity of several tetrazolium salts has been pointed out recently by K. C. Tsou and H. C. F. Su, *J. Med. Chem.*, **6**, 693 (1963).

## Experimental[11]

**Arylhydrazones of Ethyl Formate (Ia, c, and f).**—To the arylhydrazine or the corresponding hydrochloride (2 g.) 20 ml. of ethyl orthoformate was added and the mixture was heated and stirred; the ethanol was distilled as it formed. Compounds Ia and f were isolated by filtering the reaction mixture and by concentrating the filtrate. Compound Ic was obtained after removing excess EOF at reduced pressure. The crystallization solvents are listed in Table I.

**Arylhydrazones of Ethyl Acetate and Ethyl Benzoate (Ib, d, e, and g).**—A suspension of 2 g. of the arylhydrazine in 5–6 ml. of orthoester was heated with stirring, and the ethanol and the low-boiling fractions were distilled as they formed. The reaction was completed in a few minutes. If the reaction mixture did not solidify easily by cooling, excess petroleum ether (b.p. 45–70°) was added, as in the case of Ie. The crude products were recrystallized from ethanol; Id was washed thoroughly with petroleum ether, and Ig with ethyl ether before the crystallization.

**N,N'-Bis(arylamino)formamidines (II).**—The best method to obtain these compounds was by refluxing Ia, c, and f in ethanol with the equivalent amount of the corresponding arylhydrazine. These compounds were also obtained as by-products in the preparation of the arylhydrazones of ethyl formate (but starting from the arylhydrazines, not from the corresponding hydrochlorides) and could be separated in this case (Ia and f) due to their insolubility in the common organic solvents. The following compounds were obtained.

II, Ar = 4-nitrophenyl, dark brown powder, m.p. 265–265.5°; purified by washing with benzene and finally with ethanol.

*Anal.* Calcd. for $C_{13}H_{12}N_6O_4$: C, 49.37; H, 3.82. Found: C, 49.68; H, 3.68.

II, Ar = 2,4-dinitrophenyl, dark red microcrystalline powder, m.p. 229.5–230°; purified as above.

*Anal.* Calcd. for $C_{13}H_{10}N_8O_8$: C, 38.44; H, 2.48. Found: C, 38.60; H, 2.63.

II, Ar = 2-nitrophenyl, red needles, m.p. 195°; crystallizated from ethyl acetate.

*Anal.* Calcd. for $C_{13}H_{12}N_6O_4$: C, 49.37; H, 3.82. Found: C, 49.25; H, 3.54.

(11) Melting points are corrected. They were obtained with a Büchi capillary melting point apparatus. Infrared absorption spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 13 spectrophotometer.

**N-(*p*-Nitroanilino)-N′-phenylformamidine.**—An excess (15 ml.) of aniline was added to 2 g. of Ia and the mixture was heated in a steam bath for 3 hr. After cooling, a dark red precipitate was obtained; it was removed by filtration and washed with ethyl ether. The product, m.p. 206–206.5°, was obtained by crystallization from ethanol, yield 47%.

*Anal.* Calcd. for $C_{13}H_{12}N_4O_2$: C, 60.96; H, 4.72; N, 21.87. Found: C, 61.04; H, 4.91; N, 21.37.

**1-Aryl-4-arylamino-1H-1,2,4-triazolium (4) Chlorides (IV).**—A suspension of 5 g. of the arylhydrazine hydrochloride (freshly recrystallized) in 50 ml. of EOF was heated, while being stirred, by distilling the ethanol and the low-boiling fractions as they were forming. In the case of IVa and b it was not convenient to distill the formed ethanol completely. The reaction mixture was filtered at once and the residue was washed on the filter with boiling EOF, in order to remove some by-products. In the case of phenylhydrazine hydrochloride the by-product (about 0.5 g.) was identified as III, m.p. 198–198.5°, already described.[12] In the case of IVc the reaction was interrupted when 2 ml. of ethanol had distilled and reaction mixture was filtered in order to obtain the crude material. For IVd, the substance went into solution at the beginning of the reaction; as soon as a precipitate formed, the reaction mixture was filtered immediately. Compounds IV consist of crystalline colorless powders, slightly soluble in water and ethanol, practically insoluble in ether and acetone. Solvents of recrystallization are listed in Table I.

**Alkaline Degradation Products of IVa and b.**—To a saturated aqueous solution of IVa and b, respectively, an equal volume of dilute aqueous ammonia was added in small portions with stirring. A yellow-orange precipitate was formed, which was filtered at once, washed thoroughly with water, and dried *in vacuo*. The crude material was crystallized repeatedly from ethyl acetate. Compound VI (R = H) was obtained as a white crystalline powder, m.p. 163–164° dec.

*Anal.* Calcd. for $C_{14}H_{11}N_5O$: C, 66.13; H, 5.55; N, 22.03. Found: C, 66.20; H, 5.63; N, 21.79.

Under the same conditions, VI (R = 4-$CH_3$) was obtained as a white crystalline powder, m.p. 160–161° dec.

*Anal.* Calcd. for $C_{16}H_{18}N_4O$: C, 68.06; H, 6.43; N, 19.84. Found: C, 67.99; H, 6.59; N, 19.99.

By oxidative hydrolysis of compounds VI (R = H and 4-$CH_3$) the corresponding formazans VII (R = H and 4-$CH_3$) were obtained as follows: 1 ml. of 33% hydrogen peroxide was added drop by drop with stirring to 300 mg. of VI suspended in 5 ml. of 2 *N* NaOH. The suspension was allowed to react while being stirred for 15–20 hr. A red mass was formed, which was filtered and crystallized.[5] The identity of these compounds was proved by comparison of their ultraviolet spectra with those of the authentic samples.

**Hydrogenolysis of IVa and b.**—A solution of 5 g. of IVa and b, respectively, in 500 ml. of ethanol was hydrogenated with 10% palladium on carbon (100 mg.) in a low-pressure apparatus. In 5–10 hr. the theoretical amount of hydrogen was absorbed. The completion of the reaction was indicated by the fact that a sample of the solution, treated with 2 *N* NaOH, no longer gave a red color. The reaction mixture was filtered to remove the catalyst, and the ethanol was evaporated to dryness. In the case of IVa the residual oil was taken up in a mixture of ethyl ether and water (1:1). From the aqueous layer, by evaporating to dryness, aniline hydrochloride was recovered. Removing the solvent from the ether layer, an oil was obtained. The picrate of this substance (lemon yellow crystals, m.p. 160–160.5°, from water) was compared with an authentic specimen obtained from 1-phenyl-1H-1,2,4-triazole (V, R = H), prepared according to the literature.[13] The two picrates were found to give identical infrared spectra.

*Anal.* Calcd. for $C_8H_7N_3 \cdot C_6H_3N_3O_7$: C, 44.94; H, 2.69; N, 22.46. Found: C, 44.83; H, 2.60; N, 22.62.

The hydrochloride of V (R = H), m.p. 179–179.5° (subl.), was also prepared.

*Anal.* Calcd. for $C_8H_5ClN_3$: C, 52.90; H, 4.44. Found: C, 53.03; H, 4.54.

In the case of IVb, the residue was solid and was taken up in 200 ml. of water; the suspension so obtained was stirred and filtered. The residue was crystallized from diluted methanol (1:3), giving white needles, m.p. 67–67.5°. Compared as above with an authentic sample,[13] the compound proved to be the 1-*p*-tolyl-1H-1,2,4-triazole (V, R = 4-$CH_3$).

*Anal.* Calcd. for $C_9H_9N_3$: C, 67.04; H, 5.70; N, 26.41. Found: C, 67.81; H, 5.60; N, 26.51.

By evaporating the filtrate to dryness, *p*-toluidine hydrochloride was obtained.

**Acknowledgment.**—The authors are indebted to Professor V. Palma (The "Mario Negri" Institute of Pharmacological Research, Milan) for his cooperation and for making the screening data available.

(12) W. Baker, W. D. Ollis, and V. D. Poole, *J. Chem. Soc.*, 3389 (1950).
(13) G. Pellizzari, *Gazz. chim. ital.*, **24**, 222 (1894).
(14) G. Pellizzari and C. Massa, *ibid.*, **26**, 113 (1896).

## Fluorine-Containing Potential Anticancer Agents. III.[1a] Syntheses of Some Trifluoromethylpyrazolo[3,4-*d*]pyrimidines[1b]

SHOMI INOUE, HIDEO NAGANO, EDWARD A. NODIFF, AND ANDREW J. SAGGIOMO[1c]

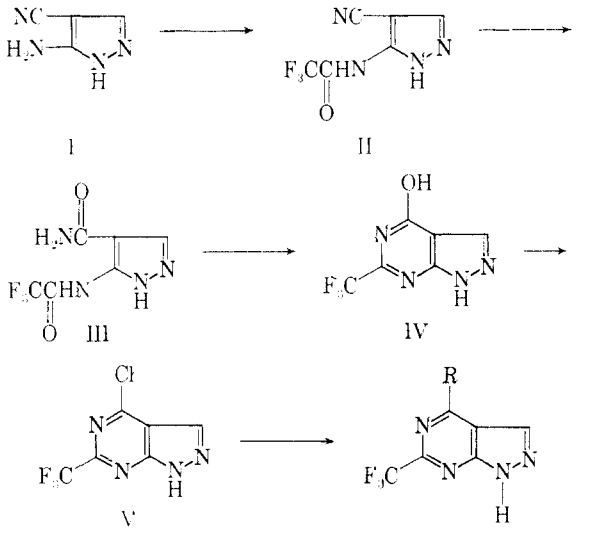
*The Research Institute of Temple University, Philadelphia, Pennsylvania 19144*

*Received May 14, 1964*

The purpose of this work was to prepare derivatives of 6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine as part of a general program in these laboratories to synthesize potential anticancer agents containing the fluoro and trifluoromethyl groups. Prior to this work fluorine-containing derivatives of the pyrazolo[3,4-*d*]-pyrimidine ring system had not been reported.

The method of synthesis outlined in Scheme I is analogous to the route used by Cheng and Robins[2]

SCHEME I




(1) (a) Paper II of this series: H. Nagano, S. Inoue, A. J. Saggiomo, and E. A. Nodiff, *J. Med. Chem.*, **7**, 215 (1964). (b) This investigation was supported by Research Grant CY-4270, from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. (c) Reprint requests should be addressed to this author.
(2) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 191 (1958).