TABLE I

CYANOHYDRIN DISSOCIATION CONSTANTS[a]

	$K_D \times 10^3$
Cyclopentanone[b]	56.0
Cyclohexanone[b]	6.00
Cholestan-3-one	5.40
Cholest-4-en-3-one[b]	38.4
Androstan-3β-ol-17-one	1.88
Allopregnan-3β-ol-20-one	148
Allopregn-7-en-3β-ol-20-one	109

[a] In 80% dioxane-water at 25.0°. [b] Ref. 1b.

TABLE II

RATES OF BROMINATION[a]

	$k \times 10^5$, $sec.^{-1}$
Cyclopentanone[b]	4.61
Cyclohexanone[b]	13.4
Cholestan-3-one	29.5
Androstan-3β-ol-17-one	48.0
Allopregnan-3β-ol-20-one acetate	6.08

[a] In 0.06M hydrogen chloride in 90% acetic acid at 25.0°. [b] Ref. 1a.

dione.[4] This relatively high reactivity of a 17-keto group may be due to the eclipsing effect of the hydrogen atoms[5] on C-12, or to relief of strain in the ketone group adjacent to the trans C/D ring fusion[6a] as its coordination number is increased to 4.[6b] The two allopregnan-20-one derivatives showed the expected large cyanohydrin dissociation constant, as do similarly substituted aliphatic ketones.[7] The order of reactivity found here is different from that found in borohydride reduction (3 > 17 > 20)[2], since although the reaction is analogous it is kinetically controlled by the attack of reagent on the carbonyl group. Similarly 17- and 20-ketones do not react with chloroform in the presence of potassium *t*-butoxide, whereas 3- and Δ^4-3-ketones react readily,[8] and this reaction is also irreversible.

The rate of bromination of androstanol-17-one is also larger than expected. Bromination proceeds *via* the enol and the increased ease of enolization of a 17-keto group may be again due to both the eclipsing effect and the strain in the exocyclic carbon–oxygen double bond, which should be relieved on forming the enol. The acyclic 20-keto group will not be subject to ring strain effects either reducing or favoring enolization and the allopregnan-20-one brominates at a rate intermediate between that of cyclopentanone and cyclohexanone.

(4) A. Ercoli and P. de Ruggieri, *J. Am. Chem. Soc.*, 75, 650 (1953).

(5) A similar effect was found in the 7-keto cyanohydrin formation, Ref. 1b.

(6) (a) *cis*-8-Methyl-1-hydrindanone has the excepted high cyanohydrin dissociation constant, Ref. 3. (b)Added in press. Fishman (*J. Am. Chem. Soc.*, 82, 6143 (1960)), has recently suggested that the conformation of ring D in 16- and 17-ketosteroids is different, and a 16-ketosteroid would accordingly be expected to have a low reactivity towards addition reactions.

(7) A. Lapworth and R. H. F. Manske, *J. Chem. Soc.*, 1976 (1930); V. Prelog and M. Kobelt, *Helv. Chim. Acta*, 32, 1187 (1949).

(8) E. Kasper and W. Wiechert, *Ber.*, 12, 2664 (1958).

EXPERIMENTAL

The rates of first order bromination and the cyanohydrin dissociation constants were determined as previously described.[1a,b]

Acknowledgment. The authors are grateful for gifts of steroids and financial assistance from Syntex, S.A., Mexico.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF PUERTO RICO AT MAYAGUEZ

Identification of Quinide in Cigarette Smoke

Y. NAKAGAWA, CHAO-HWA YANG, AND S. H. WENDER

Received November 28, 1960

Palmer[1] has reported the presence of free quinic acid (1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid) in mature, fresh cigar leaves and has shown that there is a loss of quinic acid during drying of the leaves at 80°. Nagasawa[2] has developed a microcolorimetric method for the determination of quinic acid. By this method, he found quinic acid to be present in the amount of 0.23%, dry matter, in flue-cured tobacco leaves. No previous report, however, has been made of the presence of quinic acid in cigarette smoke. A depside containing quinic acid, namely chlorogenic acid (3-caffeoylquinic acid), is a major polyphenol in tobacco leaves,[3] but has not been found as yet in smoke.

Quinide (quinic acid-γ-lactone) has not been reported in tobacco leaves, nor has it previously been reported to be in cigarette smoke.

The present paper reports our identification of quinide and of quinic acid on paper chromatograms of water-soluble extracts of absolute alcohol–acetone (1:1 v/v) solutions of mainstream cigarette smoke. On paper chromatography of pure quinide in the solvent systems of Table I, some quinic acid is produced. Therefore, whether the quinic acid found on paper chromatograms of smoke solutions was actually present as such in the smoke or was produced from the quinide during paper chromatography has not been determined.

Heating dry, authentic D-(−)-quinic acid on an oil bath up to 250° produced other compounds, as revealed by paper chromatography. One of these products has been shown to be identical with a third compound found on chromatograms of

(1) J. K. Palmer, *Science*, 126, 504 (1957).

(2) M. Nagasawa, *Bull. Agr. Chem. Soc. Japan*, 22, 205 (1958).

(3) R. A. W. Johnstone and J. R. Plimmer, *Chem. Rev.*, 59, 906 (1959).

TABLE I

R_f VALUES[a] OF QUINIC ACID, ITS DERIVATIVES, AND SMOKE PREPARATION

Compounds or Preparation	Solvent Systems[b] (1)	(2)
Quinic acid	0.24	0.21
Quinide	0.22, 0.51	0.19, 0.76
Water fraction of smoke	0.15, 0.26	0.09, 0.22
	0.51	0.76
Products of heating quinic acid	0.15, 0.31	0.07, 0.16
	0.51	0.35, 0.76

[a] Distance traveled by solvent was about 40 cm. on S and S No. 589, Red Ribbon, chromatography paper. [b] Solvent systems: (1) *n*-butyl alcohol–acetic acid–water (6:1:2 v/v/v), and (2) *n*-butyl alcohol–pyridine–saturated sodium chloride (1:1:2 v/v/v).

cigarette smoke solutions and which gives a positive Cartwright-Roberts[4] test for quinic acid-type compounds. Both quinic acid and quinide also give a positive Cartwright-Roberts test.

In several solvent systems used during the paper chromatography studies, quinide may be mistaken for shikimic acid.

EXPERIMENTAL

Preparation of smoke samples for analysis. The smoking of eight brands of cigarettes for analysis of compounds in mainstream smoke which give a positive Cartwright-Roberts test was performed by a procedure similar to the one already described for scopoletin in smoke by Yang *et al.*[5] For individual studies on paper chromatograms, one pack of each brand of cigarettes was used. For larger scale isolation and more detailed identification studies, ten packs of regular size cigarettes were smoked for each series of experiments. Cigarettes studied included Camel, Lucky Strike, Philip Morris, Old Gold Straights, L and M, Kent, and king-size Chesterfield and Salem.

Quinide and related compounds in mainstream smoke. In a typical experiment, using one pack of cigarettes, the absolute alcohol–acetone solution of the mainstream smoke was evaporated practically to dryness *in vacuo* and then dissolved in 25 ml. of water. This water solution was extracted thoroughly with pentane and then with benzene. Paper chromatograms of the pentane and of the benzene extracts failed to reveal any compounds thereon which would give a positive reaction with the Cartwright-Roberts reagent. The reagent was prepared as described by those authors[4] for the detection of quinic acid, except that potassium metaperiodate was used instead of sodium metaperiodate. Paper chromatography of the water solution of the smoke, after the pentane and benzene extractions, using S and S No. 589, Red Ribbon, chromatography paper, descending chromatography, the solvent systems of Table I, and the Cartwright-Roberts reagent as a chromogenic spray, showed that three Cartwright-Roberts-positive zones were present in each case. R_f values for each are recorded in Table I. R_f values of quinic acid, quinide, and related compounds vary considerably with variations in amounts of the compound applied to the paper and the distance traveled by the solvent. Therefore, for comparison purposes, the unknown and reference standards were run on the same sheet of chromatography paper, and concentrations of each substance spotted were kept as close as possible by estimation.

Identification studies of compounds in the three zones. The unsprayed portion of each Cartwright-Roberts-positive zone from the chromatograms of the water fraction of the mainstream smoke from cigarettes, as developed in the *n*-butyl alcohol–acetic acid–water solvent system, was cut out, sewn onto other separate sheets of chromatography paper, and each developed again in the same solvent system for identification studies.

The compound of the zone with R_f 0.15 (Table I) on paper chromatograms of the water fraction of smoke developed in *n*-butyl alcohol–acetic acid–water, was cut out, sewn onto other chromatography paper, and developed again in the same solvent system. The main zone remained at R_f 0.15, but small amounts of other unidentified zones appeared. The main zone at R_f 0.15 was compared with a compound called "FQ-III-B," one of several derivatives produced on heating dry, reference quinic acid. Comparison of paper chromatograms from thirteen different solvent systems and of ultraviolet absorption spectra showed that these two were identical. The structure of "FQ-III-B" has not yet been determined.

The compound of the zone with R_f 0.26 (Table I) on paper chromatograms of the water fraction of smoke developed in *n*-butyl alcohol–acetic acid–water was identified as quinic acid by paper chromatographic comparison with authentic, reference quinic acid in thirteen different solvent systems, and by comparison of ultraviolet absorption spectra of the reference quinic acid and of the quinic acid eluted from paper chromatograms of smoke. Authentic D-(−)-quinic acid, as purchased from several different scientific companies, showed trace amounts of two zones of impurities when streaked heavily on chromatography paper and developed in the *n*-butyl alcohol–acetic acid–water system. After two developments in this solvent system, the main zone (quinic acid) appeared to be chromatographically pure, and it continued unchanged on additional development in this solvent.

The Cartwright-Roberts-positive compound of the water extract of the smoke that had moved farthest (R_f = 0.51, Table I) on paper chromatograms developed in *n*-butyl alcohol–acetic acid–water was cut out, sewed onto a new chromatography paper, and again developed in this solvent system. The main zone was again at R_f 0.51, but some of the original compound had now changed to produce a compound with R_f about 0.22. The latter was identified as quinic acid. The major zone was identical with the major zone of pure, synthetic quinide in thirteen different solvent systems and in their ultraviolet absorption spectra.

Solvent systems used in paper chromatography identification studies. In addition to the two solvent systems listed in Table I, the following were used in the identification studies: isopropyl alcohol–pyridine–acetic acid–water (8:8:1:4 v/v/v/v); benzyl alcohol–*tert*-butyl alcohol–isopropyl alcohol–water (3:1:1:1 v/v/v/v), containing 1.8% w/v formic acid; chloroform–acetic acid–water (2:1:1 v/v); ethyl alcohol–ammonia–water (20:1:4 v/v/v); benzene–propionic acid–water (2:2:1 v/v/v); *n*-butyl alcohol–formic acid–water (8:2:1 v/v/v); 15% acetic acid–water; isoamyl alcohol–5*N* formic acid (1:1 v/v); *n*-butyl alcohol–pyridine–dioxane–water (14:4:1:1 v/v/v/v); *n*-butyl alcohol–pyridine–benzene–water (5:1:1:3 v/v/v/v); and methyl ethyl ketone–cineole–formic acid–water (50:50:20:16 v/v/v/v).

Products from the heating of quinic acid. Dry, reference quinic acid was heated, under reflux, on an oil bath. When the bath temperature reached 163°, the quinic acid started to melt, and heating was continued until the bath temperature became 250°. The heat was then turned off. After cooling, the light brown, viscous residue was dissolved in 95% ethyl alcohol and analyzed by paper chromatography, using the Cartwright-Roberts reagent. R_f values in two solvent systems of the mixture of compounds produced on heating

(4) R. A. Cartwright and E. A. H. Roberts, *Chemistry and Industry*, 230 (1955).

(5) C. H. Yang, Y. Nakagawa, and S. H. Wender, *Anal. Chem.*, 30, 2041 (1958).

the quinic acid are recorded in Table I. One of the products gave the same R_f values on paper chromatograms as quinide. The zone with R_f 0.15 on the *n*-butyl alcohol–acetic acid–water developed chromatograms of the fused quinic acid was cut out, sewn onto another chromatography paper, and developed in the benzyl alcohol–*tert*-butyl alcohol–isopropyl alcohol–water system. Zones appeared at R_f values 0.07, 0.24, and 0.53. The middle one, labeled "FQ-III-B," proved to be identical with a zone obtained on paper chromatograms of cigarette smoke as described previously.

On heating quinic acid with 6*N* hydrochloric acid for 1 hr. at 100°, Cartwright and Roberts[4] found that, in addition to quinic acid itself, three trace spots also make their appearance on paper chromatograms. One of these was indicated to be quinide.

Preparation of quinide. Quinide was prepared from quinic acid by the procedure of Panizzi and co-workers.[6]

Acknowledgment. This research was supported in part by the National Institutes of Health.

CHEMISTRY DEPARTMENT
UNIVERSITY OF OKLAHOMA
NORMAN, OKLA.

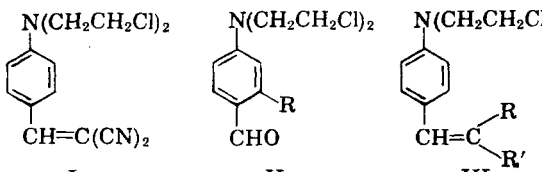
(6) L. Panizzi, M. L. Scarpati, and R. Scarpati, *Gazz. chim. ital.*, 84, 806 (1954); *Chem. Abstr.* 50, 882 (1956).

Synthesis of Potential Anticancer Agents. III. Compounds Related to *p*-[Bis(2-chloroethyl)amino]benzylidenemalononitrile[1,2]

FRANK D. POPP

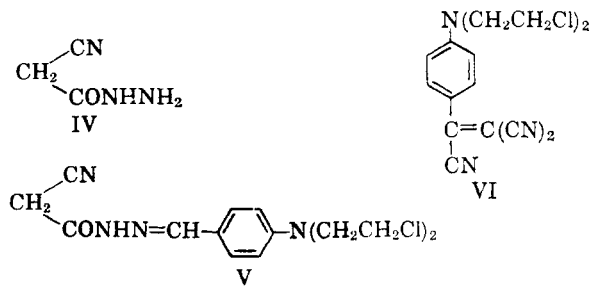
Received December 21, 1960

We have recently reported[3] the preparation of *p*-[bis(2-chloroethyl)amino]benzylidenemalononitrile (I) and several related compounds from the Knoevenagel reaction of *p*-[*N*,*N*-bis(2-chloroethyl)-amino]benzaldehyde (benzaldehyde nitrogen mustard) (II. R = H). I has been reported[4] to exhibit activity against the Dunning leukemia in rats over a wide dosage range and is currently undergoing further extensive pharmacological testing. With this great interest developing in compound I, it was decided to prepare additional related compounds. Of the compounds (III) prepared earlier,[3] those from ethyl malonate and cyanoacetamide also exhibited some activity[4] so it was decided to include analogues of these in this study. In our work with Schiff bases[1] it was found[4] that compounds prepared from 4-[bis(2-chloroethyl)amino]-*o*-tolualdehyde (II. R = CH_3) were somewhat more active than those from benzaldehyde nitrogen mustard and the same amine. This approach was therefore also tried in this series.



I II III

Malononitrile, cyanoacetamide, α-cyanoacetanilide, malonic acid, ethyl malonate, and benzoylacetonitrile were condensed with the appropriate aldehydes in dioxane using piperidine as a catalyst to give the compounds shown in Table I. Reaction of cyanoacethydrazide (IV) with benzaldehyde nitrogen mustard under a variety of conditions gave only compound V. V was very insoluble in most solvents and apparently this insolubility prevented further reaction.



The tricyanoethylene (VI) was prepared by condensation of *N*,*N*-bis(2-chloroethyl)aniline with tetracyanoethylene.[5] An attempt to convert VI to 4-(2-cyano-3-maleimidyl)-*N*,*N*-bis(2-chloroethyl)-aniline by controlled hydrolysis with concentrated hydrochloric acid[6] failed to give an identifiable product. An attempt to prepare the *N*-phenylmaleimide from *N*,*N*-bis(2-chloroethyl)aniline, sodium cyanide and *N*-phenyldichloromaleimide[6] gave only recovered starting materials.

Although the screening will be reported later in more detail,[4] it can be mentioned than the tricyano compound VI was much less active than the dicyano compound I and the compounds from α-cyanoacetanilide were less active than those from cyanoacetamide. The one arm mustards, prepared from *p*-[*N*-ethyl-*N*-(2-chloroethyl)amino]benzaldehyde, were inactive.

(1) Part II., F. D. Popp, *J. Org. Chem.*, 26, 1566(1961).

(2) This investigation was supported in part by Research Grant CY 4814 from the National Cancer Institute, U. S. Public Health Service, and in part by a Research Grant T 177 from the American Cancer Society. Presented in part before the Division of Medicinal Chemistry at the 139th Meeting of the American Chemical Society, St. Louis, Mo., March 21–30, 1961.

(3) F. D. Popp, *J. Chem. Soc.*, 5271(1960).

(4) Drs. Ralph Jones, Jr., and Leo Rane, private communication. Complete screening data on the compounds mentioned will be published elsewhere at a later date.

(5) B. C. McKusick, R. E. Heckert, T. L. Cairns, D. D. Coffman, and H. F. Mower, *J. Am. Chem. Soc.*, 80, 2806 (1958). We would like to thank Dr. McKusick for a generous sample of tetracyanoethylene.

(6) E. L. Martin, C. L. Dickinson, and J. R. Roland, *Abstracts*, 138th Meeting of the American Chemical Society, New York, Sept., 1960, page 94P. We would like to thank Dr. Martin for experimental details and samples.

(7) R. C. Elderfield, I. S. Covey, J. R. Geiduschek, W. L. Meyer, A. B. Ross, and J. H. Ross, *J. Org. Chem.*, 23, 1749 (1958).