hydroxide in 6 ml. of water. The methylated derivative was extracted with ethyl acetate and these extracts were distilled off *in vacuo*. The residue was immediately dissolved in 10 ml. of 50% ethanol, mixed with 10 ml. of 20% hydrochloric acid, and heated on a water-bath for hydrolysis. Pale yellow crystals gradually separated. After several recrystallizations, the yellow needles melted at 266–270°, and showed the properties of apigenin 5,4′-dimethyl ether. The sugar residue, therefore, was attached to position 7 of apigenin.

Isolation of Hesperidin from the Flower Petals.—Flower petals of Japanese bitter oranges, which had fallen upon the ground after pollination, were collected (June 3, 1950) at Ninomiya, Kanagawa Prefecture of the Faculty of Agriculture of the University of Tokyo. Two hundred and fifty grams of petals was twice extracted with 500-ml. portions of boiling ethanol. After the removal of the ethanol by vacuum distillation, water was added to the light-brown colored solution, and, on standing, a crystalline precipitate resulted. After 2–3 days, the precipitate was filtered, washed with acetone to remove resinous brown material, and repeatedly recrystallized from pyridine–water. The resulting colorless needles had a melting point of 250–251°. No change in melting point resulted on mixture with an authentic sample of hesperidin.

From the original mother liquor of hesperidin, no other flavonoid aglycone or glycoside was obtained.

Anal. Calcd. for $C_{28}H_{34}O_{15}$: C, 55.08; H, 5.57. Found: C, 54.75; H, 5.87.

Discussion

The results obtained with the fruit peel are contradictory to the generally accepted idea that hesperidin is contained in the fruit peels of the bitter orange. They are in sharp contrast to the report of Kolle and Gloppe who observed the presence of both neohesperidin and hesperidin in immature orange fruit peels. Whether or not neohesperidin plus hesperidin are really present in immature fruits and naringin and rhoifolin appear in their place in mature fruits, still needs experimental investigation.

In regard to the biogenetic relation between naringin and rhoifolin, there appears to be but one difference. Naringenin, the aglycon of naringin is 5,7,4′-trihydroxyflavanone; apigenin, the aglycon of rhoifolin is 5,7,4′-trihydroxyflavone. The sugar residue and its position are the same in both. There may be some possibility that in plant cells, rhoifolin may be derived from naringin by dehydrogenation; or naringin from rhoifolin by hydrogenation. Experiments are in progress to determine what glycosides are present in the fruit peel in the younger and immature stages.

Bitter orange, botanically designated as *Citrus aurantium* is now believed to be native to North India. It was transplanted from there, in ancient times, into Europe and the Far East. At present, taxonomists ordinarily consider that the European bitter orange (bitter Seville) and the Asian one represent, respectively, a variety or a horticultural form. This conclusion apparently was drawn from the morphological viewpoint, and we can find sufficient reason for it in comparison with our results. In view of the fact that the two bitter orange varieties common to Japan contain the same flavonoid glycosides in the fruit peel, they probably belong to the same phylogeny.

Acknowledgments.—We are indebted to Mr. K. Kogame, Curator of the Orchard at Ninomiya, for the flower petals used and to Dr. T. Noguchi and Mr. M. Negishi of the Chemical Laboratory of Dai-ichi Seiyaku Kaisha, Ltd., for the elementary analyses; to Dr. T. Noguchi and Dr. S. Ueda of the same laboratory for their helpful advice; and to Dr. Simon H. Wender, University of Oklahoma, for his suggestions in the preparation of this manuscript.

HONGO, TOKYO, JAPAN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Preparation of Hydantoins Containing a Cycloalkyl Substituent[1]

BY HENRY R. HENZE AND CECIL WINSTON GAYLER[2]

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The initial synthesis is reported of hydantoins having a 5-cyclopropyl, 5-cyclobutyl or 5-cyclopentyl substituent.

Despite the rather large number of 5,5-disubstituted hydantoins recorded in the literature, no examples are known of hydantoin derivatives containing a 5-cycloalkyl substituent other than cyclohexyl. The present paper reports the synthesis of six members of the series having a cyclic grouping possessing three, four or five carbon atoms.

From cyclopropanecarbonitrile, by means of the Grignard reaction, have been prepared cyclopropyl phenyl ketone and cyclopropyl isoamyl ketone. In turn, these ketones were converted into the corresponding 5-cyclopropyl-5-phenyl- and 5-isoamylhydantoins. Similarly, cyclobutyl phenyl ketone yielded 5-cyclobutyl-5-phenylhydantoin which, through catalytic hydrogenation, was converted into 5-cyclobutyl-5-cyclohexylhydantoin. Finally, methyl 2-methylcyclopentyl ketone and methyl 2,3-dimethylcyclopentyl ketone, respectively, were converted into 5,5-disubstituted hydantoins. In the first instance, higher and lower melting (in all probability the *cis* and *trans*) forms of 5-methyl-5-(2-methylcyclopentyl)-hydantoin were obtained. In the other, purification or separation of the isomers was much more difficult; although, again, a higher melting and a lower melting fraction were obtained, these do not necessarily represent pure diastereoisomers.

Experimental

Preparation of Cyclopropyl Phenyl Ketone.—Trimethylene bromide was converted into γ-bromobutyronitrile[3,4] in 56% yield, b.p. 75° (5 mm.); 208° (754 mm.); $n^{25}D$

(1) From a portion of the Ph.D. dissertation of Cecil Winston Gayler, University of Texas, June, 1943.

(2) Parke, Davis and Company Research Fellow, 1941-1943.

(3) C. Derrick and R. Hess [THIS JOURNAL, **40**, 537 (1918)] reported b.p. 100–102° (20 mm.).

(4) S. Gabriel [*Ber.*, **22**, 3336 (1889)] reported b.p. 205° (atm. press.).

1.4752; d^{25}_4 1.4810; Σ *MR* 28.18; *MR* calcd. 28.20. Subsequently, this nitrile was converted in 77% yield into cyclopropanecarbonitrile,[5,6] b.p. 35–39° (20 mm.) and 133–135° (755 mm.); $n^{25}D$ 1.4208; d^{25}_4 0.9131; Σ *MR* 18.21 (probably this summation value should be increased by about 0.7 unit because of the exaltation due to $C_3H_5C{\equiv}$); *MR* calcd. 18.70.

A Grignard reagent was prepared from interaction of 32 g. (0.2 mole) of phenyl bromide and a slight excess of magnesium and was treated with 11 g. (0.15 mole) of cyclopropanecarbonitrile in ether. The adduct was hydrolyzed with dilute hydrochloric acid. Fractionation of the product yielded 15.7 g. (78.5%) of cyclopropyl phenyl ketone[7]; redistilled b.p. 125° (20 mm.); $n^{20}D$ 1.5525; d^{25}_4 1.0456; Σ MR[8] 43.87; *MR* calcd. 44.20.

In a second preparation of this ketone, carried out essentially as before, 47 g. (0.30 mole) of phenyl bromide, 8 g. (0.33 gram atom) of magnesium and 16 g. (0.24 mole) of cyclopropanecarbonitrile gave 35 g. (99% yield) of cyclopropyl phenyl ketone.

Preparation of Cyclopropyl Isoamyl Ketone.—From 53 g. (0.35 mole) of isoamyl bromide, 9.5 g. (0.40 gram atom) of magnesium and 20 g. (0.30 mole) of cyclopropanecarbonitrile was prepared 31 g. (68% yield) of cyclopropyl isoamyl ketone, boiling at 65–70° (4 mm.). Upon redistillation: b.p. 69–70° (4 mm.); $n^{20}D$ 1.4395; d^{20}_4 0.8790; Σ MR[9] 42.29; *MR* calcd. 42.00. The 2,4-dinitrophenylhydrazone of this ketone was prepared; m.p. 95°.

Anal. Calcd. for $C_{15}H_{20}N_4O_4$: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.41; H, 6.35; N, 17.27.

Preparation of Cyclobutyl Phenyl Ketone.—Diethyl 1,1-cyclobutanedicarboxylate[10] was prepared, in 44% yield, from interaction of trimethylene dibromide, malonic ester and sodium ethylate; b.p. 106–107° (10 mm.); $n^{20}D$ 1.4335; d^{20}_4 1.0350; Σ MR[11] 50.22; *MR* calcd. 50.33.

Diethyl 1,1-cyclobutanedicarboxylate was refluxed with alcoholic potassium hydroxide solution, the potassium salt which separated was removed and treated with concentrated hydrochloric acid, and the dicarboxylic acid was obtained by ether extraction. The dicarboxylic acid was heated at 210° to accomplish decarboxylation to cyclobutanecarboxylic acid. After redistillation (68% yield) b.p. 88–90° (8 mm.) and 190° (754 mm.)[12]; $n^{20}D$ 1.4400; d^{20}_4 1.0470; Σ MR[11] 25.36; *MR* calcd. 25.20.

Cyclobutanecarboxylic acid was treated with thionyl chloride to produce cyclobutanecarbonyl chloride in 85% yield; b.p. 137° (750 mm.).[13]

Cyclobutanecarbonyl chloride reacted with benzene in the presence of anhydrous aluminum chloride to yield (35%) cyclobutyl phenyl ketone; b.p. 122–125° (8 mm.), 260–262° (750 mm.)[14]; $n^{20}D$ 1.5415; d^{20}_4 1.0467; Σ MR[11] 48.51; *MR* 48.35. Another preparation resulted in a 55% yield of this ketone.

Preparation of 5-Cyclobutyl- and 5-Cyclopropylhydantoins.—In a glass-lined steel bomb was placed 100 g. of fused acetamide,[15] 0.14–0.3 mole of potassium cyanide dissolved in its own weight of water, 0.1–0.2 mole of the appropriate cycloalkyl ketone and 0.3–0.6 mole of ammonium carbonate. The closed container was heated at 110–125° for 20 hours. After being cooled, the reaction mixture was treated with 100–200 ml. of water, causing solution of all save a portion of the hydantoin product. The solution was acidified and chilled. The crude hydantoin was removed, dissolved in 5% sodium hydroxide and filtered from some unaltered ketone; acidification of the filtrate reprecipitated the hydantoin, which was further purified by recrystallization from organic solvents. Certain data for physical properties and from analyses of these hydantoins appear in Table I. The hydantoins were obtained, in markedly poorer yields, by heating the reactants, dissolved in diluted alcohol, at 60° for 15 to 20 hours.

Preparation of 5-Cyclobutyl-5-cyclohexylhydantoin.—A suspension of 0.25 g. of the Adams platinum catalyst in 70 ml. of ethyl alcohol was shaken for 15 minutes with hydrogen under 900 mm. pressure. To the reduced catalyst was added a solution containing 10 g. of 5-cyclobutyl-5-phenylhydantoin dissolved in a mixture of 130 ml. of alcohol and 12 ml. of concentrated hydrochloric acid. This mixture was shaken with hydrogen, under 900 mm. pressure, for 12 hours; the calculated quantity of hydrogen had not been absorbed, but no further pressure drop was observed. An additional 0.25 g. of catalyst was introduced and the mixture again shaken with hydrogen for 15 hours (when the calculated amount of gas had been taken up). The catalyst was filtered off, and upon addition of water the cyclohexylhydantoin precipitated. Three crystallizations from diluted alcohol yielded 7 g. (70%) of product melting at 254–255° (cor.).

Preparation of *cis* and *trans* Forms of 5-(2-Methylcyclopentyl)-5-methylhydantoin.—A solution was prepared in fused acetamide (175 g.) of 27 g. (0.14 mole) of 2-methylcyclopentyl methyl ketone,[16] 19.5 g. (0.3 mole) of potassium cyanide and 62 g. (0.65 mole) of ammonium carbonate. The mixture was heated in a closed container at 110° for 10 hours. After being cooled, the mixture had a strong odor of the ketone and of ammonia. Considerable black material was present and was indicative of decomposition of the ketone. Water was added to precipitate the hydantoin; the latter was removed, dissolved in 5% sodium hydroxide solution; the solution was filtered, extracted with ether, and acidified to reprecipitate the hydantoin. This material was dissolved in hot acetone; upon cooling, white crystalline material separated (the mother liquor was set aside), and was recrystallized from acetone–water as white plates; 9 g.; m.p. 225–230° (cor.).[17] The acetone filtrate was evaporated to about one-half volume and diluted with an equal volume of water, causing precipitation of 4.5 g. of hydantoin material. After recrystallization from acetone–water, the product melted at 176–177° (cor.). The total weight of white needles (9.0 g.) represented a 34% yield.

Both the lower melting (*cis*) and higher melting (*trans*) forms[18] are soluble in 2% sodium hydroxide solution, from which they are reprecipitated upon acidification; heating with 4% sodium hydroxide solution results in liberation of ammonia. The higher melting form is relatively less soluble in alcohol, ether, acetone and dioxane.

In another preparation, the ketone (12.6 g.), cyanide and carbonate were dissolved in 50% alcohol and warmed at 65° for 11 hours. Upon standing at room temperature, 8 g. (41% yield) of hydantoin, melting at 210–220°, was obtained; after recrystallization, m.p. 228–230° (cor.). The alkaline filtrate was acidified and concentrated to give 8 g. (41% yield) of material melting at 175° (cor.). Analytical data for these isomeric hydantoins are placed in Table I.

Preparation of 5-(2,3-Dimethylcyclopentyl)-5-methylhydantoin.—A mixture of 28 g. (0.2 mole) of 2,3-dimethylcyclopentyl methyl ketone,[16,19] 18 g. (0.28 mole) of potas-

(5) P. Bruylants and A. Stassens [*Bull. acad. roy. Belg.*, 702 (1921); through *C. A.*, **17**, 2872 (1923)] reported b.p. 134.0–134.2° (762.5 mm.).

(6) J. Cloke, R. Anderson, J. Lachmann and G. Smith [THIS JOURNAL, **53**, 2791 (1931)] reported b.p. 35–38° (20 mm.).

(7) A. Haller and E. Benoist [*Ann. chim.*, [9] **17**, 28 (1922)] reported b.p. 235–240° (atm. press.); $n^{25}D$ 1.54335; d^{25}_4 1.0453.

(8) The summation value includes correction of 0.65 for exaltation due to conjugation of phenyl with carbonyl [K. Auwers and P. Eisenlohr, *J. prakt. Chem.*, [2] **84**, 35 (1911)], and a correction of 0.73 for exaltation due to conjugation of the cyclopropyl group with a double bond [A. A. Haller and E. Benoist, *Ann. chim.*, [9] **17**, 28 (1922)].

(9) Includes correction of 0.73 for exaltation; *cf.* ref. 8.

(10) A. W. Dox and L. Yoder [THIS JOURNAL, **43**, 680 (1921)] reported b.p. 102–105° (10 mm.).

(11) Includes correction of 0.73 for exaltation due to conjugation of cyclobutyl with carbonyl; see L. Blanchard, *Bull. soc. chim.*, [4] **49**, 279 (1931).

(12) W. Perkin [*Ber.*, **16**, 1793 (1883)] reported b.p. 193–195° (atm. press.); N. Dem'yanov and Z. Shuĭkina [*J. Gen. Chem.* (*U.S.S.R.*), **5**, 1213 (1935); through *C. A.*, **30**, 1032 (1936)] reported b.p. 189° (atm. press.).

(13) Dem'yanov, ref. 12, reported b.p. 137° (atm. press.) for the acid chloride prepared, in 84% yield, using phosphorus trichloride.

(14) Perkin, ref. 12, reported b.p. 258–260° (atm. press.).

(15) H. R. Henze and L. M. Long, THIS JOURNAL, **63**, 1936 (1941).

(16) This ketone was secured from Prof. H. L. Lochte of this department; see *ibid.*, **63**, 2975 (1941).

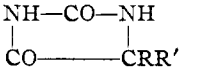
(17) This compound possesses three asymmetric carbon atoms. The substituted carbon atoms in the 1'- and 2'-positions in the cyclopentyl nucleus are capable of giving rise to *cis* and *trans* forms which would be expected to have different melting points.

(18) The *cis* form of a given stereoisomeric compound usually is less stable and has a lower melting point than the more symmetrical *trans* form; *cf.* H. Gilman, "Organic Chemistry," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1938, p. 1722.

(19) C. Neniţzescu, E. Cioranescu and I. Cantuniari, *Ber.*, **70B**, 282 (1937).

TABLE I

5-CYCLOALKYL-5-SUBSTITUTED-HYDANTOINS



R-	R'-	M.p., °C. (cor.)	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Cyclopropyl	Phenyl	211.5	56	66.65	66.44	5.60	5.62	12.93	12.75
Cyclopropyl	Isoamyl	176–177	15	62.80	62.40	8.56	8.45		
Cyclobutyl	Phenyl	234.5–235.5	85	67.90	67.61	6.13	6.00	12.17	12.01
Cyclobutyl	Cyclohexyl	254–255	70	66.07	66.00	8.53	8.65	11.86	11.42
cis-2-Methylcyclopentyl	Methyl	176–177	34	61.20	60.90	8.23	8.18		
trans-2-Methylcyclopentyl	Methyl	228–230	17	61.20	61.01	8.23	8.10		
2,3-Dimethylcyclopentyl	Methyl	145–150[a]		62.80	62.41	8.58	8.75	13.65	13.52
2,3-Dimethylcyclopentyl	Methyl	220–225[b]		62.80	62.51	8.58	8.70	13.65	13.50

[a] Fraction of lowest m.p. [b] Fraction of highest m.p.

sium cyanide and 58 g. (0.60 mole) of ammonium carbonate was dissolved in 500 ml. of 50% alcohol and warmed at 60° for 21 hours. After concentration to one-half volume and acidification, there were obtained white crystals melting at 163–175°. Considerable difficulty was experienced in recrystallizing this material, since there was a marked tendency for it to separate as an oil which finally would solidify to a non-crystalline mass. Recrystallization from a relatively large volume of diluted alcohol, with very slow cooling, gave 26 g. (62% yield) of material melting at 160–175°. The product was recrystallized in turn from diluted alcohol, acetone–water, chloroform, methanol, ether, benzene, petroleum ether and dioxane without appreciable sharpening of the melting point.[20] So the mixture was dissolved in acetone and fractionally precipitated by addition of water; from this procedure there were obtained fractions as follows:

Sample no.	M.p., °C. (cor.)	Weight, g.
1	220–225	0.5
2	210–220	1.0
3	210–215	1.2
4	175–195	6.5
5	140–180	1.5
6	145–157	1.2
7	145–150	3.0

Fractions 1 and 7 were crystallized and analyzed. The analytical data for these products, and of the other cycloalkylhydantoins prepared in this study, are listed in Table I.

AUSTIN, TEXAS

(20) This hydantoin possesses four asymmetric carbon atoms; the substituted carbon atoms at the 1'-, 2'- and 3'-positions of the cyclopentyl nucleus are capable of giving rise to *cis-cis*, *trans-trans*, *cis-trans* and *trans-cis* forms which would be expected to exhibit differences in melting point.

[CONTRIBUTION FROM THE LEDERLE LABORATORIES, AMERICAN CYANAMID COMPANY]

Experimental Chemotherapy of Tuberculosis. II. The Synthesis of Pyrazinamides and Related Compounds[1]

BY S. KUSHNER, H. DALALIAN, J. L. SANJURJO, F. L. BACH, JR., S. R. SAFIR, V. K. SMITH, JR., AND J. H. WILLIAMS

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The preparation of a large number of pyrazinamides and related compounds for antituberculous activity is described. The majority of these compounds were alkyl, aryl, acyl and heterocyclic derivatives of pyrazinamides. Thiosemicarbazones of several related pyrazines were prepared for *in vivo* testing. Of the compounds prepared, pyrazinamide was found to be more active than *p*-aminosalicylic acid in the mouse test, and was found to be clinically active in humans.

The *in vivo* antituberculous activity of nicotinamide and N-thiazolylnicotinamide, which has been reported[2] by some of us, led to further synthesis in the field of heterocyclics closely related to them. Although in the field of pyrazine chemistry several compounds have been reported to be active as analeptics,[3] antipellagric agents[4] or local anesthetics,[5] to our knowledge no study of pyrazines has been made in the chemotherapy of tuberculosis. Since the pyrazine derivatives resemble, in structure, nicotinamide with an additional nitrogen atom substituted in the ring, it was of interest to determine whether or not this similarity in structure would enable us to develop new tuberculostatic compounds. We wish to report that in Aldinamide[6] pyrazinamide, a new, comparatively non-toxic, tuberculostatic agent, with a chemotherapeutic index of 500 in the mouse, has been found. This compound has an activity several times as great as P.A.S. and seven times that of nicotinamide in the mouse.[7] Corroborative activity was obtained in the guinea pig,[8] and subsequent evaluation in the human showed it to be clinically active.[9] It should be noted that pyrazinamide is similar to nicotinamide in that it does not lend itself to chemical variation with full retention of activity. A full

(1) Presented before the Medicinal Chemistry Section, American Chemical Society, Milwaukee, Wisconsin, April, 1952.

(2) S. Kushner, H. Dalalian, R. T. Cassell, J. L. Sanjurjo, D. McKenzie and Y. SubbaRow, *J. Org. Chem.*, **13**, 834 (1948).

(3) O. Dalmer and E. Walter, German Patent 632,257 (1936).

(4) W. B. Bean and T. D. Spies, *Am. Heart J.*, **20**, 62 (1940).

(5) E. Epstein, Thesis, Polytechnic Institute of Brooklyn, 1939.

(6) Trade-mark American Cyanamid Co.

(7) L. Malone, A. Schurr, H. Lindh, D. McKenzie, J. S. Kiser and J. H. Williams, *Am. Rev. Tuber.*, **65**, 511 (1952).

(8) F. I. Dessau, R. L. Yeager, F. J. Burger and J. H. Williams, *ibid.*, **65**, 519 (1952).

(9) R. L. Yeager, W. G. C. Munroe, and F. I. Dessau, *ibid.*, **65**, 523 (1952).