2,4-dinitrophenylhydrazone of lauraldehyde, m.p. 105.5°, was obtained when the hydrolysis was carried out in the presence of 2,4-dinitrophenylhydrazine reagent.

Anal. Calcd. for $C_{36}H_{72}N_2O_2$: N, 4.96. Found: N (Kjeldahl), 4.76.

Acknowledgment.—The authors are indebted to the Procter and Gamble Company for a fellowship in support of this investigation.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY COLLEGE, CORK NATIONAL UNIVERSITY OF IRELAND]

Studies in the Pyrazole Series. I. Halogenation of the 1-Guanylpyrazoles[1]

BY FRANCIS L. SCOTT AND JOSEPH REILLY

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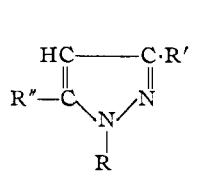
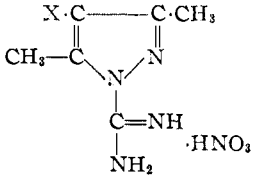
The halogenation of the 1-guanylpyrazoles and their N-substituted derivatives has been studied. With 3,5-dimethyl-1-guanylpyrazole salts and its acyl derivatives chlorine and bromine combine rapidly and reaction ceases normally at the substitution stage. Iodine is slower to react, and, under the more drastic conditions required, the process goes a step further, with a simultaneous rupture of the 1-substituent. This is lost by hydrolytic fission of the 1-imino group. With the N-naphthylcarbamyl- and N-phenylthiocarbamylguanylpyrazoles chlorine and bromine substitute initially and this is invariably accompanied by either hydrolysis or oxidation of the carbamylguanyl systems. The slower reaction of iodine has revealed the initial stages of the more complex halogenations. With the substituted naphthylcarbamylguanylpyrazole, for example, urethans result and with the thiocarbamyl, a disulfide—the heterocyclic moiety cleaving as dialkyl-4-iodopyrazole. Unambiguous synthesis of the 4-halo-1-naphthylcarbamyl- and -1-phenylthiocarbamylguanylpyrazoles confirmed that these compounds were formed initially in the halogenation of the respective carbamylguanylpyrazoles.

Variation in lability of the 1-substituent may be used as a criterion for the division of N-substituted pyrazoles into two classes, *e.g.*, while 1-alkyl (or aryl) substituted pyrazoles (I, type A) normally react[2-4] without loss of the N-substituent, pyrazoles of the 1-carbamyl[5] or 1-phenylcarbamyl[6] type (I, type B) are less stable and readily revert to the free imino compounds.



R = alkyl/aryl, type A;
(= $-CONH_2$/$-CONHC_6H_5$, type B)

I



II

This lability depends upon the reagents used and on the reaction conditions. In this present investigation we have taken a typical reaction—halogenation—as a basis for comparison and have applied it to the 1-guanylpyrazole type.

3,5-Dimethyl-1-guanylpyrazole nitrate (A, II, X = H) taken as typical of its class, has shown the 1-guanylpyrazoles to be intermediary between the 1-carbamyl and the 1-aryl types.

With chlorine and bromine (A) gives the 4-chloro- and 4-bromo-substituted derivatives (II, X = Cl, Br); with iodine and sodium acetate, deguanylation as well as substitution occurs; with stronger iodinating agents, substitution only.

Deguanylation also occurred when (A) was refluxed with sodium acetate alone in aqueous ethanolic solution—with 20–30% conversion to 3,5-dimethylpyrazole. This was a prototype of the general basic catalyzed decomposition of this type of pyrazole, taking place by one of the following schemes (next page).

Reaction by mode A results in the formation of 3,5-dimethylpyrazole (III) and urea; mode B, however, involves the intermediary formation of 3,5-dimethyl-1-carbamylpyrazole (IV), followed by its subsequent hydrolytic rupture to (III) with simultaneous evolution of carbon dioxide and ammonia.

From analogy with the hydrolytic decomposition of guanidine,[7] mode B would be the expected reaction mechanism. It was found, however, that this decomposition of the guanylpyrazole (A) occurred by the mode A formulation. This was shown by (i) the detection of urea (characterized as its dixanthyl derivative), (ii) by the absence of ammonia and carbon dioxide, in the acetate hydrolysis, and (iii) by the detection of these latter gases in the hydrolysis of the carbamylpyrazole (IV) under identical conditions.

This formulation is comparable to the mechanism of the ammonolytic and hydrazinolytic decomposition of the 1-nitroguanyl- and guanylpyrazoles, reported from this Laboratory.[8]

Deguanylation of (A) by iodine alone was also observed. A 15% conversion to 3,5-dimethyl-4-iodopyrazole took place when equimolar quantities of (A) and iodine were refluxed for eight hours in ethanolic solution. This effect is probably an acid-catalyzed hydrolysis of the guanyl group—the hydriodic acid necessary resulting from an initial iodination. This susceptibility of the guanylpyrazoles to acid hydrolysis was confirmed independently.

To define the extent of the deguanylating effect, and in order to determine its mechanism, the halogenation of substituted guanylpyrazoles of the general type (V) was undertaken.

(1) Presented in part at the XII International Conclave of Chemistry, New York, 1951, Section 12.

(2) L. Knorr, *Ann.*, **279**, 232 (1894).

(3) L. Knorr and A. Blank, *Ber.*, **18**, 311 (1885).

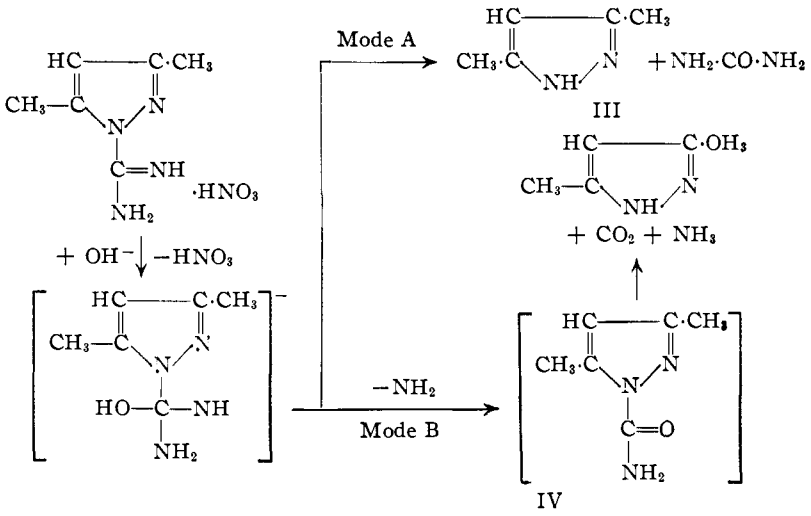
(4) L. Balbiano, *Gazz. chim. ital.*, **18**, 358 (1888).

(5) T. Posner, *Ber.*, **34**, 3980 (1901); K. von Auwers and B. Ottens, *ibid.*, **55**, 2072 (1952); A. Dornow and K. Peterlien, *ibid.*, **82**, 2571 (1949).

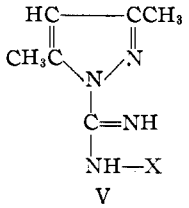
(6) A. S. Wheeler, R. D. Norton and F. P. Brooks, THIS JOURNAL, **50**, 2488, 3390 (1928).

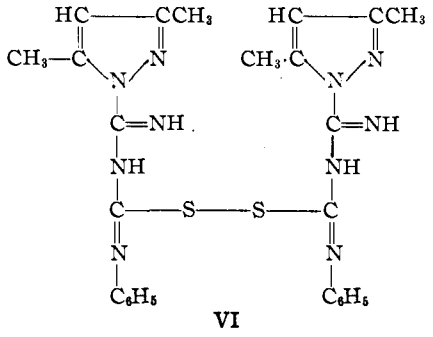
(7) J. Bell, *J. Chem. Soc.*, 1213 (1926); G. Laude, *Compt. rend.*, **208**, 1848 (1939).

(8) F. L. Scott, M. T. Kennedy and J. Reilly, *Nature*, **169**, 72 (1952).



Initially, 3,5-dimethyl-1-(N-1′-naphthylcarbamyl)-guanylpyrazole (C, *i.e.*, V, X = $CONHC_{10}H_7(1')$,) and 3,5-dimethyl-1-(N-phenylthiocarbamyl)-guanylpyrazole (D, *i.e.*, V, X = $-CSNHC_6H_5$) were halogenated, but the reactions proved


V


VI

complicated, *e.g.*, (C) with one molar quantity of chlorine affords the desired halo compound, while with excess halogen rupture of the molecule occurs. The points of cleavage are, probably, at the naphthylureido carbon atom and at the imino carbon atom. Such fission was only observed to a very slight extent during bromination while, with all modes of iodination attempted, due to the prolonged treatment necessary, similar decomposition occurred.

With (D) the halogens behave in a manner analogous to that in which they react with thiourea itself. The least active of them, iodine, oxidizes (D) to bis-(3,5-dimethyl-1-guanylpyrazolyl-(N-phenyl)-iminomethyl) disulfide (VI). The other halogens form this disulfide initially, but the reaction proceeds further.

With bromine, 3,5-dimethyl-1-(N-*p*-toluenesulfonyl)-guanylpyrazole (E), *i.e.*, V, X = $SO_2-C_6H_4-CH_3-p$) gave the 4-bromo compound. With chlorine, decomposition as well as substitution occurred and some *p*-toluenesulfonyl chloride was liberated. As the reactions of (D) and (C) with the halogens proved complex, independent syntheses of 3,5-dimethyl-4-halo-1-(N-1′-naphthylcarbamyl- and N-phenylthiocarbamyl)-guanylpyrazoles were undertaken. (A) was initially halogenated, the haloguanylpyrazole salts obtained converted to the respective free bases and finally reaction of these free bases with reagents of the isocyanate and isothiocyanate types afforded the desired materials.

The reaction of 3,5-dimethyl-1-(N-benzoyl)-guanylpyrazole (F, *i.e.*, V, X = COC_6H_5) afforded reasonable indication of the mechanism of the iodine-produced deguanylation. With chlorine and bromine the 4-chloro and 4-bromo-substituted materials, respectively, resulted, whereas iodine alone, or in the presence of sodium acetate, effected hydrolytic fission of the molecule, 3,5-dimethyl-4-iodopyrazole and benzoylurea being obtained.

Even with stronger iodinating agents this same rupture resulted, reaction by the Tucker technique taking 16 minutes, the others requiring many hours for completion. The isolation of benzoylurea indicated the hydrolytic nature of this deguanylation. This facile, acid-catalyzed hydrolysis is in contrast to that of aminoguanidine.[9] While the guanylpyrazole (A) is susceptible to basic and acidic hydrolysis, (F) is inactive toward reagents of the basic type, being quantitatively recovered unchanged from eight hours refluxing with sodium acetate in ethanolic solution. Similarly sodium and alcohol reduced the material without severing the 1-substituent. Finally, it exhibited none of the electrophilic activity of (A) toward hydrazine hydrate or phenylhydrazine.

The small quantity of hydriodic acid formed during the iodination of (F) stopped the hydrolysis at the benzoylurea stage. With greater quantities of acid, the molecule was cleaved quantitatively into benzoic acid and 3,5-dimethylpyrazole hydrochloride—the rapid, acid-catalyzed hydrolysis of the guanyl group in (A) taking place simultaneously with the cleavage of the benzamido linkage.

Experimental[10,11]

3,5-Dimethyl-1-guanylpyrazole Nitrate (A).—(A) was prepared[12] with average yields of *ca.* 70% by refluxing equimolar quantities of acetylacetone and aminoguanidine nitrate in aqueous ethanolic solution for three hours.

Anal. Calcd. for $C_6H_{11}N_5O_3$: C, 35.8; H, 5.5; N, 34.8. Found: C, 35.5; H, 5.3; N, 34.6.

Picrate.—Recrystallized from aqueous ethanol and separated as small needles of m.p. 207–208.5°.

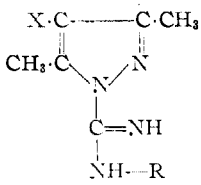
Anal. Calcd. for $C_{12}H_{13}N_7O_7$: C, 39.2; H, 3.5; N, 26.7. Found: C, 39.5; H, 3.5; N, 26.5.

Flavianate.—Recrystallized in fine yellow needles (from aqueous ethanol), m.p. 194–195°. The salt is of the general type: $(Base)_2$ (flavianic acid).

(9) E. Lieber and G. B. L. Smith, THIS JOURNAL, **59**, 2283 (1937).
(10) All melting points are uncorrected.
(11) Microanalyses by Drs. Weiler and Strauss, Oxford, England.
(12) J. Thiele and E. Dralle, *Ann.*, **302**, 275 (1898).

TABLE I

SUBSTITUTED 3,5-DIMETHYL-1-GUANYLPYRAZOLES



Ref. letter	Substituted-1-guanylpyrazoles	Formula	Yield, %	M.p., °C.	Analyses, % Calculated C	H	N		Found C	H	N	
C	X = H, R = 1′-Naphthylcarbamyl[f]	$C_{17}H_{17}N_5O$	90	140–141	66.4	5.5	22.8	.....	66.8	5.3	22.8	.....
D	X = H, R = Phenylthiocarbamyl[g]	$C_{13}H_{15}N_5S$	82	106	56.9	5.5	25.6	S 11.7	57.0	5.6	25.8	S 11.4
E	X = H, R = *p*-Toluenesulfonyl[h]	$C_{18}H_{16}N_4O_2S$	13	141–142	53.2	5.8	19.1	S 11.0	53.7	5.8	19.0	S 10.8
F	X = H, R = Benzoyl[i]	$C_{13}H_{14}N_4O$	73	111–112	64.4	5.7	23.1	.....	64.4	5.7	22.9	.....
G	X = Cl, R = H[j]	$C_6H_9ClN_4$	50–60	54–56	..	...	32.5	Cl 20.6	..	...	32.2	Cl 20.7
H	X = Br, R = H[k]	$C_6H_9BrN_4$[a]	50–60	66–67	30.6	4.7	23.8	Br 34.0	30.9	4.9	24.4	Br 34.0
J	X = Cl, R = Phenylthiocarbamyl[l]	$C_{13}H_{14}ClN_5S$	68	136–137	50.7	4.6	22.6	S 10.4[b]	51.0	4.6	23.8	S 9.6[c]
K	X = Br, R = Phenylthiocarbamyl[m]	$C_{13}H_{14}BrN_5S$	64	139–140	44.3	4.0	21.6	S 9.1[d]	44.5	4.1	20.0	S 9.0[e]
L	X = Cl, R = 1′-Naphthylcarbamyl[n]	$C_{17}H_{16}ClN_5O$	90	157–158	59.7	4.7	20.5	Cl 10.4	59.9	4.7	20.6	Cl 10.8

[a] Analysis corresponds to a monohydrate. [b] Cl (calcd). 11.5. [c] Cl (found) 11.4. [d] Br (calcd.) 22.7. [e] Br (found) 21.7. [f] Substance B + 1-naphthyl isocyanate, mixed in benzene–petroleum ether soln. [g] Substance B + C_6H_5NCS, in ethanolic soln. [h,i] Usual Schotten–Baumann technique with substance A + respective acid chloride. [j] By chlorination of A (see Table II, followed by treatment with excess concd. KOH soln. [k] Bromination of A and treatment with excess KOH soln. [l] G + C_6H_5NCS (in ethanol). [m] H + C_6H_5NCS (in ethanol). [n] G + 1′-naphthyl isocyanate (in petroleum ether).

Anal. Calcd. for $C_{22}H_{26}N_{10}O_8S\cdot H_2O$: N, 23.0; S, 5.3. Found: N, 23.1; S, 5.3.

3,5-Dimethyl-1-guanylpyrazole (B).—The inertness of the salts of (B) toward reagents of the isocyanate, isothiocyanate and sulfonyl halide types rendered the preparation of the free base essential as a preliminary to the production of pyrazoles containing substituted carbamylguanyl, thiocarbamylguanyl and sulfonylguanyl groups in the 1-position. Thiele had previously failed to isolate this unstable material but we found it possible to prepare it as follows. To 20 g. of (A) dissolved in 100 ml. of cold water was added 100 ml. of concentrated (90 g. in 100 ml.) potassium hydroxide solution. Despite the fact that the temperature was maintained below 20° during the addition, some deguanylation occurred. The 3,5-dimethylpyrazole thus produced was, however, removed from the guanylpyrazole free base during the ensuing steps. The free base, some of which separated from the very strongly alkaline medium as a yellowish oil, was removed from the solution by extracting it, six times, with 40-ml. portions of ether. The ethereal extracts after drying were evaporated in a stream of air and a white mass of crude material (m.p. 58–64°) remained. On recrystallization from dry benzene, it was obtained as white needles of characteristic odor, of m.p. 70–71°; yield 80% (10.9 g.). On repetition, yields of from 80–90% were obtained.

Anal. Calcd. for $C_6H_{10}N_4$: C, 52.2; H, 7.3; N, 40.6. Found: C, 52.6; H, 7.3; N, 40.0.

Substituted Guanylpyrazoles.—The substances given in Table I were prepared during these investigations, either as starting materials for halogenations, or, after halogenation, as intermediates or possible end substances.

Two techniques are summarized in Table I, (i) the preparation of some haloguanylpyrazole free bases in a manner exactly analogous to that of the parent free base (B) (see above), and (ii) the reaction of these free bases with some reagents of the isothiocyanate and isocyanate types.

The preparation of 3,5-dimethyl-1-(N-phenylthiocarbamyl)-guanylpyrazole (D) is typical of the latter method of synthesis.

To 3 g. of (A) in 10 ml. of absolute ethanol was added dropwise an equimolar quantity, 2.6 ml., of phenyl isothiocyanate. The solution turned a deep yellow and after boiling for five minutes was allowed to stand for 48 hours. A yellow crystalline mass of crude (D) (4.8 g., 82% yield) separated. This was obtained after recrystallization from absolute ethanol in fine, highly refractory, yellow granules of m.p. 105–106.5°.

Halogenations[13]

(i) **Chlorinations.**—With the parent substance (A) and its N-benzoyl derivative (F, Table I), chlorine smoothly substitutes in the 4-position of the heterocyclic ring. With C, D and E (Table I) complex reactions result.

(13) For summary see Table II.

(ii) **Brominations.**—A, C and F reacted normally. A (D) behaved in an anomalous manner, its reaction will be described.

(a) **Bromination (Rapid Addition).**—To 1 g. of (B) in carbon tetrachloride (10 ml.) was added 0.22 ml. of bromine. A red solid was immediately precipitated and the whole became a semi-solid. A further 20 ml. of carbon tetrachloride was added and the thick solution vigorously boiled for a few moments. It was then allowed to stand overnight, filtered and dried. The yield (1.3 g.) is practically quantitative. The crude material, melting at 126–128°, was 4-bromo-3,5-dimethyl-1-(N-phenylthiocarbamyl)-guanylpyrazole. On recrystallization from aqueous ethanol a profound change apparently took place for the white solid obtained was impure and melted over a range of 180–204°. Repeated recrystallization from both 95% and absolute ethanol resulted in the purification of the material in fine, white needles of m.p. 221–223°.

Anal. Calcd. for $C_{34}H_{30}Br_3N_{14}S_2$: C, 43.5; H, 3.1; Br, 25.6; N, 20.9; S, 6.8. Found: C, 43.7; H, 3.2; Br, 25.7; N, 20.6; S, 6.9.

If the bromine (0.23 ml.) was added dropwise with constant stirring to 1 g. of (D) dissolved in 10 ml. of chloroform, each droplet initially caused the precipitation of a red, sticky solid which almost completely redissolved on shaking. The clear solution thus obtained was then allowed to stand, a colorless oil began to separate out, and white fumes of HBr were evolved. On removing the chloroform in a stream of air, a white solid, 1.66 g. of m.p. *ca.* 220–224°, was obtained. On repeated recrystallizations from absolute ethanol it was obtained as long, white needles of m.p. 233° and was bis-(3,5-dimethyl-1-guanylpyrazolyl-(N-phenyl)-iminomethyl) disulfide.

Anal. Calcd. for $C_{26}H_{28}N_{10}S_2$: C, 57.6; H, 4.8; N, 25.8; S, 11.8. Found: C, 57.2; H, 4.8; N, 26.0; S, 12.1.

(iii) **Iodinations.**—The reactions were still more complex, the more prolonged treatment required to overcome the lack of reactivity of the halogen causing profound changes in the molecule. Thus, (A) or the sulfate of (B) with equimolar quantities of iodine and sodium acetate in aqueous ethanolic solution, after eight hours refluxing affords 3,5-dimethyl-4-iodopyrazole, m.p. 134°,[14] in high yield.

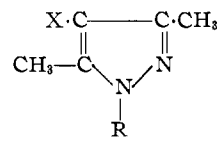
Similarly, while reaction of (A) with iodine only effects 15% conversion to 3,5-dimethyl-4-iodopyrazole, iodination by the Tucker[15] technique results in a 48% yield of the iodopyrazole and a 12% yield of 3,5-dimethyl-4-iodo-1-guanylpyrazole (isolated as picrate). The reaction of (A) with iodine monochloride is complex, thus: To 5 g. of (A) dissolved in 50 ml. of glacial acetic acid was added 4.04 g. of iodine monochloride in 60 ml. of the same solvent. The deep violet-red color of the solution faded to a light yellow-

(14) Reported m.p. 137°. See G. T. Morgan and I. Ackerman, *J. Chem. Soc.*, 1312 (1923).

(15) S. H. Tucker, *ibid.*, 546 (1926).

TABLE II

HALOGENOGUANYLPYRAZOLES



Compound no.	Product (from)	Reaction	M.p., °C.	Analyses, % Calculated C	H	N		Found C	H	N	
1	X = Cl, R = Guanyl nitrate	A + Cl_2 (boiling aqueous soln.)	180	30.5	4.0	29.6	Cl 15.0	30.5	4.2	29.9	Cl 15.2
2	X = Br, R = Guanyl nitrate	A + Br_2 (cold aqueous solution)	171	26.3	3.6	25.0	Br 28.5	26.0	3.5	25.0	Br 28.2
3	X = I, R = H	A + I_2 + NaO·Ac (aq. soln.)	134	27.0	3.1	12.6	I 57.2	26.6	3.3	12.2	I 57.5
4	X = I, R = Guanyl nitrate[f]	A + ICl (HOAc)[j]; A + KI + KIO_3(HO·Ac)[j]	224	..	...	22.1	I 25.1	..	...	21.6	I 25.4
5	X = Br, R = N-1′-Naphthylcarbamylguanyl	C + Br_2 (boiling CCl_4 solution)	182[g]	52.8	4.1	18.1	Br 20.7	52.3	4.3	18.3	Br 20.8
6	X = Br, R = N-Phenylthiocarbamylguanyl[a]	D + Br_2 (CCl_4 soln. rapid addition)	221–223[a]	43.5	3.2	20.9	Br 25.6[b]	43.7	3.2	20.6	Br 25.7[c]
7	Bis-(3,5-dimethyl-1-guanylpyrazoyl)-(N-phenyl)-iminomethyl disulfide[k]	D + Br_2 (CCl_4 soln. slow addn.)	233	57.6	4.8	25.8	S 11.8	57.2	4.8	26.0	S 12.1
8	X = Br, R = N-*p*-Toluenesulfonylguanyl[h]	E + Br_2 (CCl_4 soln. room temp.)	158–159	42.1	4.0	15.1	Br 21.5[d]	42.5	3.9	15.1	Br 20.7[e]
9	X = Cl, R = N-Benzoylguanyl	F + Cl_2 ($CHCl_3$ soln. room temp.)	127–128	56.4	4.7	20.3	Cl 12.8	56.9	4.5	20.4	Cl 12.8
10	X = Br, R = N-Benzoylguanyl	F + Br_2 (warm aqueous ethanol soln.)	136	48.6	4.1	17.4	Br 24.9	48.7	4.2	16.7	Br 24.6
11	Benzoylurea + (4-iodo-3,5-dimethylpyrazole)[i]	F + iodinating agents[c]	210	58.5	4.9	15.9	.....	58.5	4.8	17.1	.....
12	Compound of mol. form. $C_{11}H_{13}I_4N_4$[j]	A + ICl (HOAc)	232	18.6	1.8	7.9	I 71.6	18.9	1.8	7.9	I 71.5

[a] Initially K (Table I) was formed. This crude K changed on recryst. from aq. ethanol. This analysis is for the (unidentified) resultant solid. [b] S (calcd.) 6.8. [c] S (found) 6.9. [d] S (calcd.) 8.6. [e] S (found) 8.5. [f] Isolated as picrate. [g] M.p. varies with length and rate of heating. [h] Orientation proved by "tosylation" of H (Table I). [i] With KI, KIO_3 and HOAc, or I_2 and NaOAc, or ICl in HO·Ac. [j] See following Experimental section for details of the other solids obtained in these reactions. [k] Also detained from D and I_2.

red after a few moments refluxing. Heating was continued for 30 minutes to ensure completion of the reaction and then the solution was poured into water. A pinkish-white precipitate of m.p. *ca.* 223° separated as a fine amorphous powder. This was filtered, dried and extracted with absolute alcohol to remove any 3,5-dimethyl-4-iodopyrazole present. (Alcoholic extracts gave 3,5-dimethyl-4-iodopyrazole picrate, m.p. 191–192°.)

Anal. Calcd. for $C_{11}H_{10}IN_5O_7$: I, 28.2; N, 15.6. Found: I, 27.3; N, 14.9.

The residual pinkish-white solid tended to decompose when heated in contact with any of the usual solvents, and melted sharply at 232°.

Anal. Calcd. for $C_{11}H_{13}I_4N_4$: C, 18.6; H, 1.8; I, 71.6; N, 7.9. Found: C, 18.9; H, 1.8; I, 71.5; N, 7.9.

It corresponds to a compound of empirical formula $C_{11}H_{13}I_4N_4$. It was not a periodo derivative of 3,5-dimethyl-4-iodopyrazole (chloroplatinate, m.p. 215–220°) as it formed light-red hexagons of a chloroplatinate which fused at 241°.

In a repetition of this experiment, a portion of the reaction solution was concentrated, alcohol and aqueous picric acid were added. 3,5-Dimethyl-4-iodo-1-guanylpyrazole picrate (m.p. 224° after recrystallization from absolute ethanol) separated.

Anal. Calcd. for $C_{12}H_{12}IN_7O_7$: I, 25.1; N, 22.1. Found: I, 25.4; N, 21.6.

Reaction of (A) with Sodium Acetate.—To 2 g. of (A) dissolved in 30 ml. of water was added 1.36 g. (0.01 mole) of crystalline sodium acetate and the whole was refluxed. After one hour heating, the characteristic odor of 3,5-dimethylpyrazole was apparent, but no ammonia evolution could be detected. Refluxing was continued for a further seven hours, during which time the quantity of 3,5-dimethylpyrazole produced increased but no ammonia was evolved. After standing at room temperature for 24 hours, the solution was extracted five times with 30-ml. portions of ether, which extracts deposited 0.16 g. of crude 3,5-dimethylpyrazole of m.p. 101–103°. From the aqueous mother liquor 0.33 g. of 3,5-dimethyl-1-guanylpyrazole picrate and 0.25 g. of an unidentified picrate (decomposing over 260–360°) was obtained. Urea was recognized as one of the products of the decomposition of (A) as follows. Two grams of (A) was treated with sodium acetate as above. After all the 3,5-dimethylpyrazole had been removed with ether, the aqueous solution was evaporated to dryness. Extraction of the dry residue with glacial acetic acid and treatment of the extracts with a 7% glacial acetic acid solution of xanthydrol[16] resulted in the separation of crude dixanthyl urea (1.5 g.), m.p. 252–258°.

A hydrolysis experiment cognate to the one described above was similarly performed with 3,5-dimethyl-1-carbamylpyrazole. The product was again 3,5-dimethylpyrazole. With the 1-carbamylpyrazole, however, the boiling sodium acetate solution caused evolution of both ammonia and carbon dioxide in considerable quantities.

The material (C) proved sensitive to hydrolytic rupture, *e.g.*, with sodium acetate in alcoholic solution and eight hours refluxing, 3,5-dimethylpyrazole and 1-naphthylurethan were formed. With iodine, 3,5-dimethyl-4-iodopyrazole and 1-naphthylurethan were obtained. With iodine chloride in acetic acid, 3,5-dimethyl-4-iodopyrazole and 3,5-dimethyl-4-iodo-1-guanylpyrazole, together with an oxidation product of 1-naphthyl isocyanate were obtained. Similarly, hydrochloric acid caused rupture of the molecule into a mixture of 3,5-dimethylpyrazole hydrochloride, 1-naphthylurea and di-1-naphthylurea. With phenylhydrazine, 1-phenyl-4-(1′-naphthyl)-semicarbazide separated from a boiling alcoholic solution of one molar quantity of reactants. This sensitivity of (C) rendered the halogenation of the material complex. The 4-halo derivatives were decomposed by light or by traces of water in the reaction mixture.

Iodination of (D) under the mildest conditions yielded a disulfide while with (F) hydrolysis was again observed, thus: To 0.87 g. of (F) dissolved in 15 ml. of absolute ethanol was added slowly a mixture of 1 g. of iodine and 2.5 g. of crystalline sodium acetate, dissolved in 15 ml. of absolute ethanol. The solution was refluxed for 30 minutes to complete reaction and benzoylurea was isolated from the mother liquor on concentration as flaky white crystals of m.p. 208–210° (reported m.p. 214°).

Anal. Calcd. for $C_8H_8N_2O_2$: C, 58.5; H, 4.9; N, 15.9. Found: C, 58.5; H, 4.8; N, 17.1.

Similar hydrolytic rupture of (F), with iodine alone, or iodine chloride in glacial acetic acid, or even with potassium iodide, potassium iodate and acetic acid (the fastest reaction:

(16) Prepared from xanthone by Holleman's method, see A. F. Holleman, "Organic Syntheses," Coll. Vol. I, J. Wiley and Sons, Inc., New York, N. Y., 1946, p. 554.

16 minutes) always afforded benzoylurea and 3,5-dimethyl-4-iodopyrazole.

Hydrolysis of F, with excess 10% hydrochloric acid, resulted in a quantitative yield of benzoic acid and 3,5-dimethylpyrazole hydrochloride.

Hydrazine hydrate and phenylhydrazine did not react with (F) and it displayed a similar negligible electrophilic activity with sodium acetate after eight hours refluxing, and also with one molar quantities of sodium and alcohol at room temperatures. In large excess (20 molar quantities of sodium) these latter reagents under reflux reduced (F) to a pyrazoline without however any observed formation of benzoylurea.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF OKLAHOMA]

The Isolation and Identification of Quercetin and Isoquercitrin from Black Currants (*Ribes nigrum*)[1]

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Black currants, *Ribes nigrum*, have been reported by both the guinea pig test and clinical assay method to possess a relatively high "vitamin P" activity, which is generally attributed to the flavonoid compounds present. This paper reports the isolation and identification of quercetin (3,3',4',5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from dried black currants.

Introduction

Bacharach, *et al.*,[2,3] have reported that a concentrate from black currants, *Ribes nigrum*, possesses a relatively high "vitamin P" activity. Apparently the tests were made using a concentrate prepared from black currants in a manner described by Pollard.[4] In a later study, Pollard[5] reports the isolation of a crystalline substance from black currants. From the properties exhibited by this substance, Pollard postulates that it is a flavonol. No positive identification, however, was achieved.

To date, to our knowledge, no flavonoid compounds have been reported as having been identified from black currants or their concentrates. The present paper reports the isolation and identification of quercetin (3,3',4',5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from dried black currants, *Ribes nigrum*.

For the isolation of the flavonoid compounds the following unit processes were employed: boiling water extraction, ion exchange chromatography, concentration and drying *in vacuo*, extraction with hot anhydrous acetone, adsorption chromatography, precipitation as the lead salt, decomposition of the lead salt, neutralization of a filtrate by ion exchange, and recrystallization from water. In the identification procedure, use was made of paper partition chromatography, melting point determinations, ultraviolet absorption spectra and preparation of a derivative by methylation and hydrolysis.

Experimental

Twenty pounds of dried black currants, *Ribes nigrum*, was soaked in water at room temperature for three hours. This caused the currants to swell and increased the effectiveness of the wet grinder, through which they were processed as the next step.[6] The discharged extract from the wet grinder was diluted to 20 gal. with distilled water and digested for one hour at the boiling point. The extract was cooled to approximately 70° and filtered. The residue was discarded and the filtrate allowed to cool to room temperature. The cooled extract was then passed over ion exchange columns at the rate of one gal./hr. for each column. Four columns were used with 5 gal. of extract passed over each. A column consisted of a glass tube 6 × 100 cm. drawn to an outlet at one end filled to a depth of 80 cm. with Amberlite IRC-50(H) (Rohm and Haas, Philadelphia, Pa.). The columns containing the material adsorbed from the extract were each washed with 2 gal. of distilled water to remove the sugar, and the effluent and washings were discarded.[7] The adsorbed material, containing the flavonoids present, was then eluted from the columns with 500 ml. of 95% ethyl alcohol for each of the four columns. This eluate was then taken to dryness *in vacuo* using a resin pot immersed in a hot water-bath. The pulverized residue was then transferred to a Soxhlet extractor and extracted for 36 hr. with 500 ml. of anhydrous acetone. This acetone extract, after cooling to room temperature, was passed through a chromatographic column, 38 × 220 mm., containing a bed of magnesol (Food Machinery and Chemical Corp., Westvaco Chemical Division, New York), 100 mm. deep. One hundred ml. of the extract seemed to be the optimum load for a column. Material in the extract was adsorbed on the magnesol, giving a band approximately 10 mm. deep and yellow in visible light. The chromatogram was developed with ethyl acetate saturated with water.[8] A band, yellow in both visible and ultraviolet light, moved off the column first. The portions containing this band were combined and concentrated *in vacuo* to 5 ml., and 50 ml. of pentane was added to the cooled solution. The solid was removed by centrifugation and identified as quercetin. The yield at this point was 20 mg. Yields reported in this paper, however, do not represent the actual flavonoid content of the currants, as purity for qualitative analysis was the object of the research, and no effort was made to determine the exact quantity of each flavonoid as present in the black currants.

By paper partition chromatography, the solid showed R_f values of 0.06 in 15% acetic acid, 0.77 in butanol–acetic acid–water (40-10-50%, by volume), and 0.42 in 60% acetic acid, and no separation from authentic quercetin by mixed paper chromatography in each of the solvents mentioned.

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