arabinose, by the orcinol method of Brown[4]). If unknown sugars are present in this fraction they can be adsorbed directly on a borate column and analyzed individually by the method of Khym and Zill.[7] Essentially, quantitative recoveries of the sugars and the uronic acids were obtained.

The identity of the peaks was determined by column runs on the individual uronic acids and by isolation and characterization of the uronic acid in the peak. Eluate fractions were evaporated to dryness *in vacuo* and characterized as the benzimidazole derivative according to the procedure of Lohmar, Dimler, Moore, and Link.[8]

(7) J. X. Khym and L. P. Zill, THIS JOURNAL, **73**, 2399 (1951); **74**, 2090 (1952).

(8) R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol. Chem.*, **143**, 551 (1942).

BIOLOGY DIVISION J. X. KHYM
OAK RIDGE NATIONAL LABORATORY D. G. DOHERTY
OAK RIDGE, TENNESSEE

RECEIVED MARCH 29, 1952

3,5-DIAMINO-*AS*-TRIAZINES AS INHIBITORS OF LACTIC ACID BACTERIA AND PLASMODIA

Sir:

The inhibitory activity of various 2,4-diaminopyrimidines on the growth of *Lactobacillus casei* was interpreted as a competitive antagonism between these substances and folic acid.[1] The formal structural analogy between certain 5-substituted diaminopyrimidines and the known antimalarial chlorguanide (I) led to the suggestions that the diaminopyrimidines might have antimalarial properties and that both the biguanide and pyrimidine might act on plasmodia as antagonists of folic acid and related compounds.[2] Further investigations of the antimalarial properties of the pyrimidines culminated in the synthesis of 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine (II)[3] which has a minimum effective dose[4] of about 0.03 mg./kg. corresponding to activity of 60 times chloroguanide against *Plasmodium gallinaceum* and 200 times against *P. berghei*. Furthermore, evidence relating the antimalarial activity of both types of substance to the folic acid metabolism of the parasite was forthcoming.[5,6]

Recent investigations have shown that chlorguanide (I) is converted to 2,4-diamino-1-*p*-chlorophenyl-6,6-dimethyl-1,6-dihydro-1,3,5-triazine (III) *in vivo*,[7] and that this metabolite is an active antimalarial[7,8] and an antagonist of the folic acid group of vitamins in microbiological studies.[8] It is apparent that a real structural analogy between II and III exists and is determinative of the similarity of biological behavior of I and II.

(1) G. H. Hitchings, G. B. Elion, H. VanderWerff and E. A. Falco, *J. Biol. Chem.*, **174**, 765 (1948).

(2) E. A. Falco, G. H. Hitchings, P. B. Russell, and H. VanderWerff, *Nature*, **164**, 107 (1949).

(3) E. A. Falco, L. G. Goodwin, G. H. Hitchings, I. M. Rollo and P. B. Russell, *Brit. J. Pharm.*, **6**, 185 (1951).

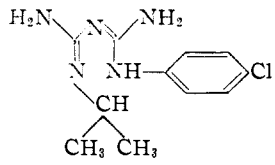
(4) I. M. Rollo, *Nature*, **168**, 332 (1951).

(5) J. Greenberg and E. M. Richeson, *J. Pharm. Exp. Therap.*, **99**, 444 (1950).

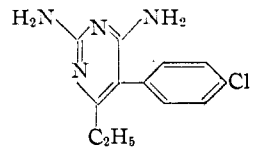
(6) J. Greenberg, *Proc. Soc. Exper. Biol. Med.*, **71**, 306 (1949).

(7) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi and F. L. Rose, *Nature*, **168**, 1080 (1951).

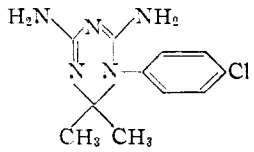
(8) E. J. Modest, G. E. Foley, M. M. Pechet and S. Farber, THIS JOURNAL, **74**, 855 (1952).



I

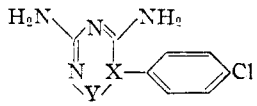


II



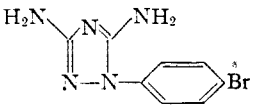
III

The structural feature common to II and III is the unit IV. The significance of this structural unit, in various heterocyclic systems, has been under investigation in these laboratories for some time. For example, 3,5-diamino-1-*p*-bromophenyl-1,2,4-triazole (*p*-bromophenylguanazole) (V) and several 3,5-diamino-6-phenyl-1,2,4-triazines (VI) have been synthesized, and their biological activities have been investigated. (**3,5-Diamino-1-*p*-bromophenyltriazole**, m.p. 210°. *Anal.* Calcd. for $C_8H_8N_5Br$: C, 36.8; H, 3.2. Found: C, 36.6; H, 3.3. **3,5-Diamino-6-(3′4′-dichlorophenyl)-1,2,-4-triazine**, m.p. 219–220°. *Anal.* Calcd. for $C_9H_7N_5Cl_2$: C, 42.2; H, 2.7. Found: C, 42.4; H, 3.0. **3,5-Diamino-6-(4′-chlorophenyl)-1,2,4-triazine**, m.p. 218–220°. Calcd. for $C_9H_8N_5Cl$: C, 48.9; H, 3.6. Found: C, 48.6; H, 3.6.)

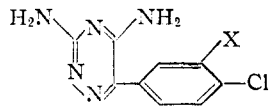


IV

X = N, C, C—CH_2, C—O
Y = C, N



V



VI

X = Cl or H

Biologically the asymmetrical triazine (VI, X = H) closely resembles the pyrimidine (II) and the symmetrical dihydrotriazine (III). Thus it is a competitive antagonist of folic and folinic acids in the growth of *L. casei* and of folinic acid (synthetic "Leucovorin") in the growth of *Leuconostoc citrovorum*, with an inhibition index in the latter system of approximately 20,000 (at half-maximal growth). With VI, as with II and III, the minimum concentration for inhibition of *Streptococcus faecalis* is several hundred-fold greater when folinic acid is supplied than when the organisms are grown with folic acid. This is in contrast to the finding with *L. casei* where folic and folinic acids are essentially equivalent over a wide range of concentration of the inhibitor. The antimalarial activities of VI and its congeners are quite high. The activity of the 3,4-dichlorophenyl analog of VI against *P. berghei* is 230 times that of chlorguanide; it is, however, less active than chlorguanide in *P. gallinaceum* infections.

The biological activities of the guanazole (V) are

generally weaker than those of the triazines and perhaps qualitatively different. Thus V is only a weak inhibitor of *L. casei* and its activity is not clearly blocked by folic acid. Furthermore, its antimalarial activity is minimal.

The above observations lend support to the previously expressed views concerning the relationship between antifolic acid and antimalarial activity[2] and further delineate the chemical structures with which this activity is associated.

G. H. HITCHINGS
THE WELLCOME RESEARCH LABORATORIES A. MAGGIOLO
TUCKAHOE, NEW YORK P. B. RUSSELL
H. VANDERWERFF
THE WELLCOME LABORATORIES OF
TROPICAL MEDICINE I. M. ROLLO
LONDON, ENGLAND

RECEIVED APRIL 29, 1952

STEROIDAL SAPOGENINS. V.[1] ENZYMATIC HYDROLYSIS OF STEROIDAL SAPONINS

Sir:

It is well known that plants containing cardioactive glycosides have enzyme systems which can partially hydrolyze these substances.[2] We have recently found that leaves of some Agave and Yucca species which contain steroidal saponins have similar enzyme systems. In contrast to the enzymes acting on cardio-active glycosides, those found in the sapogenaceous plants tested cleave the saponin substrate to the sapogenin aglycone and component sugars or polysaccharides.

An enzyme preparation was obtained by grinding 2 kg. of frozen *Agave toumeyana*[3] leaves, followed by extraction with water at 10°. The filtered extract was shaken with benzene I and the aqueous layer containing the enzyme fraction held at 4°. The leaf residue remaining from the aqueous extraction was refluxed with 95% ethanol to extract residual saponins. The ethanol solution was concentrated, diluted with water, extracted with benzene II, and the aqueous saponin fraction heated on the steam bath to drive off residual alcohol.

The aqueous enzyme and saponin solutions were combined, adjusted to *p*H 5.25 and held at 37° for 90 hours. The resultant turbid suspension was extracted first with a mixture of 90% benzene–10% ethanol III to recover enzyme hydrolyzed sapogenins and then with butanol IV to obtain unhydrolyzed or partially hydrolyzed saponins.

Only a trace of sapogenin was found in the prehydrolysis benzene extracts I and II. The extract III was concentrated and chromatographed on activated alumina. Using methods presented in detail elsewhere[4] the following sapogenins were isolated: (1) hecogenin (isoallospirostan-12-one-3β-ol), m.p. Kofler 255–260°, on Wolf–Kishner reduction gave tigogenin (isoallospirostan-3β-ol) m.p. 203–205°, yield 3.9 g.; (2) manogenin (isoallospirostan-12-one-2α,3β-diol) m.p. 240–244°, yield 1.3 g., on Wolf–Kishner reduction gave gitogenin (isoallospirostan-2α-3β-diol) m.p. 268–270°. Infrared spectra of all sapogenins and their Wolf–Kishner reduction products were identical (with exception of manogenin which showed slight divergencies) with those of authentic specimens. Hecogenin and manogenin had a carbonyl peak in the infrared at 1708–1710 cm.$^{-1}$, absent in reduced products. Manogenin also showed conjugated carbonyl at 1678 cm.$^{-1}$ from which it is deduced that 10–20% of the 9$^{(11)}$ dehydro component was present.

From IV after subsequent acid hydrolysis and chromatography were isolated 2.4 g. of sapogenin similar in composition to those found in III, indicating that the enzymatic hydrolysis was 78–80% complete under our experimental conditions.

In a duplicate experiment, the sapogenins were isolated entirely by means of acid hydrolysis. The yield of total sapogenin was similar and the sapogenins isolated identical with the enzymatic hydrolysis.

Using methods similar to those described above, acid or enzyme hydrolysis of *Agave serulata*[5] gave hecogenin, manogenin and small quantities of the 9$^{(11)}$-dehydro analogs of both sapogenins; an unidentified Yucca species[6] yielded with both methods sarsasapogenin (spirostan-3β-ol) and an unidentified Dioscorea species gave diosgenin (Δ^5-isospirosten-3β-ol) in both cases.

In agreement with Marker, *et al.*,[7] we find that steroidal sapogenins occur in plants only as glycosides as evidenced by the fact that free sapogenin could not be found prior to hydrolysis. Our data are not in accordance with the views of Marker and Lopez[8] that the spiroketal side chain and the 12-keto group in sapogenins are artifacts produced by acid hydrolysis. The fact that the much milder enzyme hydrolysis gives the same products as acid cleavage tends to cast doubt on Marker's hypothesis. Our previous findings[9] based on infrared studies of saponins have also shown that the structure of the steroidal moiety of saponins and their sapogenins derived by acid hydrolysis are in all probability identical.

Because of the mild conditions used, tars, resins and pigments produced by acid hydrolysis are not found in the enzymatic method, thus simplifying the isolation of purified sapogenins.

The distribution, occurrence, and physico-chemical properties of the enzyme systems found in sapogenaceous plants are being studied and will be reported in detail at a later date.

EASTERN REGIONAL RESEARCH LABORATORY
BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY
AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE MERLE M. KRIDER
PHILADELPHIA 18, PA. MONROE E. WALL

RECEIVED MAY 9, 1952

(1) Paper IV of this series submitted for publication in THIS JOURNAL. This work was done as part of a coöperative arrangement between the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, and the National Institutes of Health, Federal Security Administration.

(2) For pertinent references see A. Stoll and associates, *Helv. Chim. Acta*, 1933–1951, and W. A. Jacobs, *Physiol. Rev.*, **13**, 222 (1933).

(3) Collected by H. S. Gentry, #S 2046, Superior, Arizona, December, 1951.

(4) M. E. Wall, M. M. Krider, E. S. Rothman and C. R. Eddy, *J. Biol. Chem.*, in press.

(5) Collected by H. S. Gentry, #1865, San Ignacio, Baja California, November, 1951.

(6) Collected by H. S. Gentry, #2161, Jecabasco, Mexico, December, 1951.

(7) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(8) R. E. Marker and J. Lopez, *ibid.*, **69** 2390 (1947).

(9) E. S. Rothman, M. E. Wall and H. Walens, *ibid.*, in press.