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2,4-DINITROPHENYLOSAZONES FROM CORIOSE AND D-GLUCOSE[1]

FORMATION IN MINERAL ACID BY DIFFERENT ROUTES

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Abstracts—1-Deoxycoriose 2,4-dinitrophenylosazone **2** was obtained in strong mineral acid as the only crystalline osazone of coriose **1**. Experimental evidence was obtained for the mechanism of formation of **2** *via* 1-deoxyosone **10**. The structure of D-glucose 2,4-dinitrophenylosazone produced analogously was proved to be **6**, and the isolation of 2,4-dinitroaniline from the mother liquor is in accord with the mechanism of normal phenylosazone formation. Excess reagent did not cause further reaction down the chain of the sugars, and the large downfield shift of an imino proton in each osazone, showing that a chelate ring is formed between two phenylhydrazine moieties in these osazones in spite of the strong chelation between *a*-imino and *o*-nitro group in 2,4-dinitrophenylhydrazine.

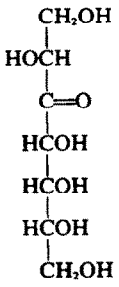
While phenylosazone from aldoses and 2-ketoses have been extensively investigated, no example of a phenylosazone from 3-ketose has been presented although there have been several 3-ketoses, i.e., naturally occurring coriose (D-*altro*-3-heptulose) **1**,[2] synthetic D-*manno*-3-heptulose[3] and D-*gluco*-3-heptulose,[4] and also some 3-hexuloses[5] and 3-pentuloses.[6] Osazone should be an important tool, if it is obtained, for the analysis of these 3-ketoses because of the difficulty of their analyses caused by the complexity of the equilibrium states as shown by GLC of TMS derivatives.[4,7] There is also a problem concerning the possibility that 2,3- as well as 3,4- osazones might be produced from 3-ketoses concurrently. There has already been arguments of the possibility of the osazone formation at C-2 and C-3 besides at C-1 and C-2 from 2-ketoses.[8] 2,4-Dinitrophenylosazones from D-glucose and some other monosaccharides were reported without detailed proof of the structures and purities.[9] These osazones were reported to be produced quantitatively in 2N HCl while it has been reported that sugar phenylosazones are produced with difficulty in strong acid.[10] Studies of the structures and the mechanisms of formation of 2,4-dinitro phenylosazones from coriose and D-glucose have been carried out by the present work.

We first attempted to prepare coriose phenylosazone under various reaction conditions including the regular method,[11] but the experiments gave tar and partial recovery of coriose. Reactions of coriose with α-methylphenylhydrazine, p-nitrophenylhydrazine, and p-bromophenylhydrazine resulted in recovery of the major portion of coriose.

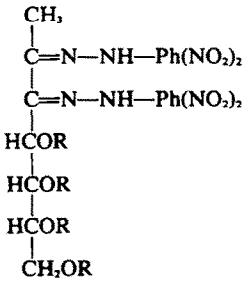
A crystalline osazone **2** from coriose was finally obtained by the reaction with 2,4-dinitrophenylhydrazine in hot 2N HCl or 85% phosphoric acid. After repeated recrystallization, the main product, m.p. 233°, analyzed as $C_{19}H_{20}O_{12}N_8$. The UV absorptions, $\lambda_{max}^{CHCl_3}$ (log ϵ) 405 (4·59) and 445 mμ (4·59), are at slightly longer wavelength than those of glyoxal bis-2,4-dinitrophenylhydrazone, $\lambda_{max}^{CHCl_3}$ (log ϵ) 390 (4·63) and 437 mμ (4·65). This osazone yielded a crystalline tetraacetate **3**, $C_{27}H_{28}O_{16}N_8$, m.p. 214–215° (dec), which shows five singlets of acetyl or vinyl methyl groups in the NMR spectrum. Among the protons at C-4 ~ C-7, which were confirmed by the NMDR experiment, C-4 proton shows unusually large downfield shift to δ 7·04.

Although this osazone is poorly soluble, its NMR spectrum in DMSO-D_6 shows a methyl signal at δ 2·34 which is assignable to $CH_3—\underset{|}{C}=N—$. Therefore, the acetate would have four acetyl groups, and the osazone should have the acyclic structure **3**, or one of the cyclic forms, **3a**, **3b**, **3c** and **3d** etc. Among them, **3** is most favoured because of absence of the amide absorption in the IR spectrum.

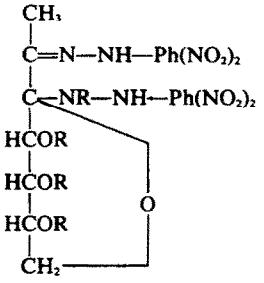
The large downfield shift of C-4 proton in the NMR spectrum of the acetate suggests that this might be a vinyl proton. However, this possibility is excluded by the IR spectrum of **3** which shows absence of aliphatic double bond, and also by the



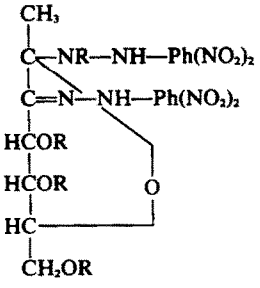
1



2: R = H
3: R = Ac



2a: R = H
3a: R = Ac



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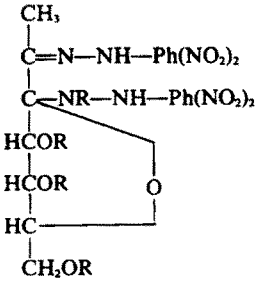
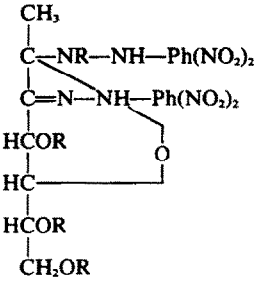
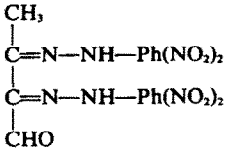
3b: R = Ac



2c: R = H
3c: R = Ac



2d: R = H
3d: R = Ac



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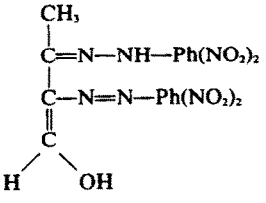
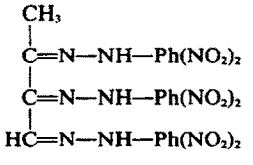


4a



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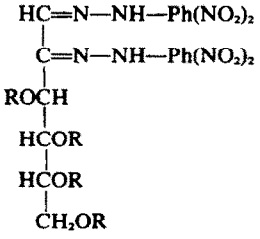
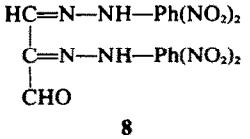


6: R = H
7: R = Ac



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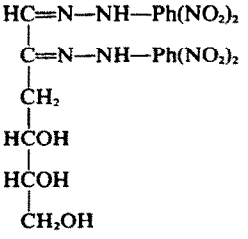

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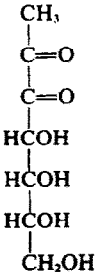

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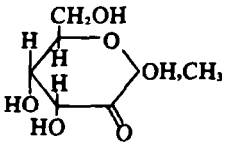
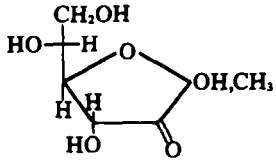
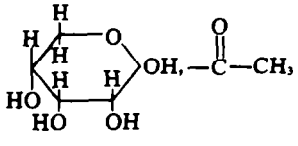


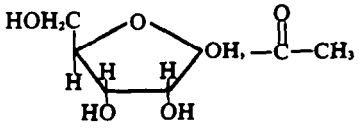
10a



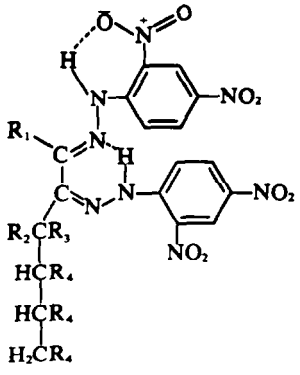
10b



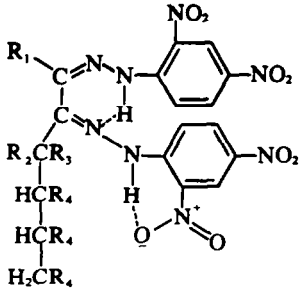
10c



10d



2a: $R_1 = CH_3$, $R_2 = H$, $R_3 = R_4 = OH$
3a: $R_1 = CH_3$, $R_2 = H$, $R_3 = R_4 = OAc$
6a: $R_1 = H$, $R_2 = R_4 = OH$, $R_3 = H$
7a: $R_1 = H$, $R_2 = R_4 = OAc$, $R_3 = H$



2b: $R_1 = CH_3$, $R_2 = H$, $R_3 = R_4 = OH$
3b: $R_1 = CH_3$, $R_2 = H$, $R_3 = R_4 = OAc$
6b: $R_1 = H$, $R_2 = R_4 = OH$, $R_3 = H$
7b: $R_1 = H$, $R_2 = R_4 = OAc$, $R_3 = H$

periodic acid oxidation upon which the osazone consumed 3 moles of periodic acid in dioxane, and gave a precipitate, m.p. 263° (dec), which was analyzed as $C_{16}H_{12}O_9N_8$. The mass spectrum shows M^+ peak (*m/e* 460) and *m/e* 442 peak which are in accord with structure **4**. This oxidation product afforded tri-2,4-dinitrophenylhydrazone **5**, m.p. 289–292° (dec), $C_{22}H_{16}O_{12}N_{12}$, on the reaction with 2,4-dinitrophenylhydrazine. However, **4** is presumed to exist as enol **4a** in the solid state, since only weak absorption of the carbonyl group is shown in its IR spectrum (Nujol), and the absorption in the region of enol (1605 or 1594 cm^{-1}) is stronger than that of **2**.

The results of periodic acid oxidation, which is analogous to the oxidation of ordinary phenylosazone,[12] coupled with the characterization of the acetate as acyclic as described above, also favour the acyclic structure **2** of 1-deoxycoriose 2,4-dinitrophenylosazone rather than the cyclic structures, such as **2a** etc. The large downfield shift of C-4 proton in NMR spectrum of **3** is attributable to the influence by the 2,4-dinitrophenylhydrazine moieties.

As for the formerly reported 2,4-dinitrophenylosazones from D-glucose and some other monosaccharides, which were presented based on the elemental analyses,[9] we have found that the crude products are contaminated by marked amounts of tarry products. However, the structure of the main product of the reaction of D-glucose with 2,4-dinitrophenylhydrazine in 2N HCl has been proved to be **6** as follows. Recrystallized D-glucose 2,4-dinitrophenylosazone, $C_{18}H_{16}O_{12}N_4$, m.p. 270–271°, shows absorption, $\lambda_{max}^{CHCl_3}$ (log ϵ) 390 (4·50) and 440 mμ (4·53), which is analogous to that of glyoxal bis-2,4-dinitrophenylhydrazone. Upon acetylation, tetraacetate **7**, $C_{26}H_{26}O_{16}N_8$, m.p. 137–140° (dec) was produced. The NMR spectrum (60 MHz, $CDCl_3$) shows four singlets of acetyl groups, well dissolved protons of C-3 ~ C-6, and downfield shift of C-3 proton to δ 6·72. This downfield shift is regarded as being due to the influence by the 2,4-dinitrophenylhydrazine moieties as the osazone structure is supported by the periodic acid oxidation on which a product, $C_{15}H_{10}O_9N_8$, m.p. 260–264°, whose structure is regarded as **8**, was obtained.

The mechanism of the formation of (**6**) is apparently different from that of (**2**), since otherwise the main product from D-glucose would be 3-deoxyosazone (**9**). Detection of (**9**) in the crude product and the mother liquor of (**6**) was attempted without success.

A probable mechanism of the formation of **2** is that proceeds *via* 1-deoxyosone **10** which might be formed in acid like 3-deoxyosone which was postulated as the intermediate in acid catalyzed production of furfurals from sugars.[13] The second mechanism would involve dehydration at C-2 of coriose 2,4-dinitrophenylhydrazone and attack by the second 2,4-dinitrophenylhydrazine molecule at the cation at C-2, followed by dehydration at C-1 ~ C-2. The experimental results have been found in accord with the first mechanism. Coriose was treated with hot 2N HCl, and after cooling, a cooled solution of 2,4-dinitrophenylhydrazine in 2N HCl was added. The precipitate which appeared immediately was identified as **2**. Isolation of the intermediate 1-deoxyosone was also attempted by deionizing the solution resulted on the treatment of coriose with hot 2N HCl, and further purifying by preparative PC. The obtained syrup which was shown almost pure by PC and GLC of TMS ether, showed in the NMR spectrum a methyl signal at δ 1·50. Therefore, 1-deoxycoriosone syrup would mainly be present as a cyclic form **10a** or **10b**, as the methyl proton in the tautomers **10c**, **10d** and **10** should shift lower. This syrup produced **2** with 2,4-dinitrophenylhydrazine reagent.

2,4-Dinitroaniline, which should be produced if the osazones are formed by the route analogous to that of ordinary phenylosazone, as represented by Weygand's mechanism,[12] was isolated from the mother liquor of **6**, but was not detected in the mother liquor of **2**. The reaction producing **6** was also conducted in nitrogen atmosphere, and passing oxygen, to find whether the oxidation of the intermediate product as proposed for one of the possible mechanisms of phenylosazone formation[12] occurred, but the results were always the same.

The yield of **6** on the reaction with 2 moles of the reagent was markedly lower than the yield with 3 moles of the reagent. The yield of **2** on the reaction with 3 moles of the reagent was somewhat higher than that with 2 moles of the reagent, but the difference was not so marked as that of **6**. These results also support the above shown difference of the mechanism between **2** and **6**.

Upon the reaction of coriose and D-glucose with 4 moles of 2,4-dinitrophenylhydrazine, no further reaction down the chain of the sugar was not observed at least to an appreciable extent. If the chelate ring formation between the two phenylhydrazine moieties which has been considered to stop further reaction in ordinary phenylosazones[12] is also working in 2,4-dinitrophenylosazone, this chelation must be stronger than the chelate ring formed between α-imino and o-nitro group in each 2,4-dinitrophenylhydrazine moiety. In the NMR spectrum (90 MHz, DMSO-D_6) of **2**, two peaks of the chelated NH protons are observed at δ 11·09 and δ 13·83. The NMR spectrum of the acetate **3** shows the imino protons at δ 11·46 and 12·72 in $CDCl_3$. The chelations of both of these protons were strong enough to resist the exchange with deuterium when D_2O was added to the solutions. The higher imino proton is regarded as that of the chelate ring including o-NO_2 group as the NMR spectra of mono-2,4-dinitrophenylhydrazones usually show the imino proton around this region.[14] The lower proton would then only be attributable to the one in the chelate ring between the two phenylhydrazine moieties, as ordinary phenylosazones show the downfield shift of the chelated NH proton to approximately the same region.[15] The chelated structure of **2** is then shown as either **2a** or **2b**, and the structure of **3** would be **3a** or **3b**.

The NMR spectrum of D-glucose 2,4-dinitrophenylosazone shows the two imino protons at δ 11·80 and 13·60, and the acetate **7** shows them at δ 11·48 and 12·67. Their structures would be represented by **6a** or **6b**, and **7a** or **7b**.

EXPERIMENTAL

TLC was run on silicic acid acc. to Stahl (E. Merck, 0·25 mm) in $CHCl_3$–MeOH (9:1) or benzene–AcOEt (7:3) unless otherwise specified. Elution chromatography was run on Mallinckrodt's silicic acid (100 mesh). Preparative PC was carried out on thick filter papers (40 × 40 × 0·7 cm) with n-BuOH–pyridine–H_2O (6:4:3). Deoxyosone was detected by spraying 2,4-dinitrophenylhydrazine reagent, and also sodium metaperiodate-benzidine[16] on a few strips cut from the thick filter paper. The area of the same R_f value on the residual filter paper was then cut off, and extracted with MeOH. TMS derivatives were prepared with trimethylchlorosilane–hexamethyldisilazane–pyridine (1:2:10, v/v/v). GLC was carried out with Shimadzu 5A gas chromatograph equipped with FID using a glass column (2 m × 3 mm i.d.) packed with 3% OV-17 on 80–100 mesh Chromosorb W treated with HMDS. R_{Glu} shows the retention time relative to α-D-glucose. NMR spectra were recorded with Hitachi R-22 at 90 MHz, Varian A-60 at 60 MHz, and Jeol JNM-4H-100 at 100 MHz with tetramethylsilane as the internal standard. Mass spectra were determined with Hitachi RMU-6D mass spectrometer at 80 eV using all-glass inlet system.

1-*Deoxycoriose* 2,4-*dinitrophenylosazone* **2**. (a) With 3 moles of the reagent in 2N HCl: A soln of coriose (50 mg) in water (2 ml) was added to a warm soln of 2,4-dinitrophenylhydrazine (165 mg) in 2N HCl (7 ml), and the mixture was warmed in a boiling water-bath for 3 h. Ppt was filtered from the warm reaction mixture, and dried *in vacuo* (100·1 mg). This ppt was recrystallized from dioxane–MeOH or AcOEt, m.p. 214–215° (dec). $\lambda_{max}^{CHCl_3}$ (log ϵ) 405 (4·59), 445 mμ (4·59). NMR (90 MHz, DMSO-D_6) δ 2·34 (s, CH_3—C=N—), 11·09 (s, NH), 13·83 (s, NH). Found: C, 41·70; H, 3·71; N, 20·02. $C_{19}H_{20}O_{12}N_8$ requires: C, 41·31; H, 3·65; N, 20·28%. The crude ppt showed an almost single orange spot on TLC

(CH_2Cl_2–MeOH, 20:1) when the reaction was stopped within 1 h.

A small amount of additional crop was precipitated out on cooling. The mother liquor was neutralized with Na_2CO_3 to precipitate unreacted 2,4-dinitrophenylhydrazine. These precipitates and the mother liquors showed no spot of 2,4-dinitroaniline on TLC.

(b) With 2 moles of the reagent in 2N HCl: An analogous reaction as above was carried out with coriose (50 mg) and 2,4-dinitrophenylhydrazine (110 mg) in 2N HCl (20 ml). The ppt from the warm reaction mixture was identified with the above product by TLC and IR spectra (dried *in vacuo*, 96·5 mg).

(c) With 4 moles of the reagent in 2N HCl: A soln of coriose (50 mg) in water (1 ml) was added to a soln of 2,4-dinitrophenylhydrazine (220 mg) and the mixed soln was warmed for 2 h. Ppt was filtered from the warm soln and dried *in vacuo* (99 mg). The product was identified by TLC and IR spectra with the product from the reactions with 2 and 3 moles of the reagent.

(d) With 3 moles of the reagent in phosphoric acid: A soln of coriose (50 mg) in water (1 ml) was added to a warm soln of 2,4-dinitrophenylhydrazine (150 mg) in ortho-phosphoric acid (s.g. 1·70) and the mixture was warmed for 20 min on a boiling water-bath. Ppt was filtered from the warm soln and dried *in vacuo* (80 mg). This product was shown on TLC contaminated by fast moving spots, and identified with **2** after repeated recrystallization.

1-*Deoxycoriose* 2,4-*dinitrophenylosazone tetraacetate* **3**. A mixture of **2** (198 mg), Ac_2O (2·5 ml) and pyridine (2·5 ml) was left overnight, and the resultant soln was treated with ice-water (45 ml). Ppt was filtered, dried (59 mg), dissolved in benzene (20 ml), and chromatographed on a silicic acid column (2·7 × 17 cm) eluting with benzene–tetrahydrofuran (99·5:0·5 at first, secondly 99:1, and finally 97:3). The fractions which were eluted with 3% tetrahydrofuran, and showed a single spot of the main product were collected and the solvent was distilled. The residue was recrystallized from benzene (80·3 mg), and then from $CHCl_3$–benzene–petroleum ether m.p. 204° (dec) (59·0 mg). $\lambda_{max}^{CHCl_3}$ (log ϵ) 392 (4·62) and 438 mμ (4·62). NMR (100 MHz, $CDCl_3$) δ 1·87, 1·94, 2·02, 2·33, 2·44 (Ac and $CH_3-\underset{|}{C}=N-$), 4·1 ~ 4·5 (m, C_7–H × 2), 5·2 ~ 5·4 (m, C_6–H), 5·68 (t, $J_1 = J_2 = 6$ Hz, C_5–H), 7·04 (d, $J = 5$ Hz, C_4–H), 7·95 and 8·42 (ABq, $J = 9·5$ Hz, C_6–H and C_5–H), 8·27 and 8·47 (ABq, $J = 10$ Hz, C_6–H and C_5–H) (the doublets at 8·42 and 8·47 show long-range coupling $J = 2$ Hz with C_3–H at 9·18), 11·46 and 12·72 (strongly chelated NH, observed even after warming with D_2O). Found: C, 44·97; H, 4·01; N, 15·75. $C_{27}H_{28}O_{16}N_8$ requires: C, 45·00; H, 3·92; N, 15·55%.

Periodic acid oxidation of 1-*deoxycoriose* 2,4-*dinitrophenylosazone* **2**. A soln of **2** (79 mg) in dioxane (15 ml) was treated with $HIO_4.2H_2O$ (114·2 mg, 98·3%) in water (2 ml) for 62 h. Orange ppt appeared in a few minutes. The excess periodic acid was determined in 0·5 ml portions of the soln with 0·01 N sodium arsenite by titration in sodium bicarbonate soln of the iodine liberated after addition of potassium iodide. The residual total oxidizing capacity was estimated in acidic soln with 0·1 N sodium thiosulphate. The consumption per mole of **2** corresponded to 3·06 moles. The dark red ppt was collected by centrifuge, washed with EtOH and water successively, and dried (26 mg). The product **4** was purified by passing a short silicic acid column, and by recrystallization from dioxane, m.p. 263° (dec). $\lambda_{max}^{CHCl_3}$ (log ϵ) 265 (4·41) and 458–470 mμ (4·60). ν_{max}^{Nujol} 1670 cm^{-1}. Mass spectrum: M^+ 460, *m/e* 442 ($M^+ - 18$). Found: C, 41·83; H, 2·65; N, 24·39. $C_{16}H_{12}O_9N_8$ requires: C, 41·74; H, 2·63; N, 24·34%.

Tri-2,4-dinitrophenylhydrazone of the oxidation product. A soln of **4** (24 mg) in dimethylformamide (8 ml) was added to a soln of 2,4-dinitrophenylhydrazine (10·6 mg) in dimethylformamide (1 ml) containing a drop of conc HCl, and the mixed soln was left stand 2 days. Ppt was filtered, washed with 2N HCl, water and EtOH successively. This ppt was then dissolved in CH_2Cl_2 (100 ml) and chromatographed on a column of silicic acid (1·2 × 8 cm) eluting with CH_2Cl_2. The fractions which showed an orange–red spot on TLC were concentrated and deposited crystals of **5** were filtered (8 mg), m.p. 289–292° (dec). $\lambda_{max}^{CHCl_3}$ (log ϵ) 270 (4·50) Mass spectrum: *m/e* 457 ($M^+ - 183$). Found: C, 41·39; H, 2·57; N, 26·09. $C_{22}H_{16}O_{12}N_{12}$ requires: C, 41·26; H, 2·52; N, 26·25%. Dissolution of this product in morpholine resulted in a greyish-brown soln, which on standing gave colourless crystals which are regarded as those of a morpholine adduct, as **5** was recovered from the CH_2Cl_2 soln of these colourless crystals.

D-*Glucose* 2,4-*dinitrophenylosazone* **6**. (a) With 3 moles of the reagent in 2N HCl: A soln of D-glucose (500 mg) in water (2 ml) was added to a hot soln of 2,4-dinitrophenylhydrazine (1·65 g) in 2N HCl (100 ml) and the mixture was warmed in a boiling water-bath for 7·5 h. The dark red ppt was filtered from the warm soln, dried (1·37 g) and recrystallized from AcOEt, m.p. 270 ~ 271°. $\lambda_{max}^{CHCl_3}$ (log ϵ) 390 (4·50) and 440 mμ (4·53). Found: C, 40·30; H, 3·33; N, 20·39. $C_{18}H_{18}O_{12}N_4$ requires: C, 40·15; H, 3·38; N, 20·81%. Upon cooling, 114 mg of ppt which showed on TLC a main spot identical with the initial ppt was obtained. The mother liquor was neutralized with Na_2CO_3, and orange–red ppt was filtered off (200 mg). This ppt was shown on TLC (silicic acid, AcOEt–benzene 1:1) to be a mixture of 2,4-dinitrophenylhydrazine (main) and **6**. The neutralized mother liquor was extracted with ether to yield a small amount of orange residue which showed on TLC the spot corresponding to 2,4-dinitroaniline. After recrystallization from EtOH, this product was identified with 2,4-dinitroaniline (99·5 mg).

(b) With 2 moles of the reagent in 2N HCl: An analogous reaction as (a) was carried out with coriose (500 mg) and 2,4-dinitrophenylhydrazine (1·10 g) in 2N HCl (61 ml) for 7·5 h. The first ppt was identified with the above product (1·069 g). The second ppt, deposited on cooling (80·4 mg), showed the main spot on TLC identical with the first ppt. The mother liquor was neutralized with $NaHCO_3$, but immediate precipitation was not observed.

(c) With 3 moles of the reagent in phosphoric acid: A soln of D-glucose (99 mg) in water (1 ml) was added to a warm soln of 2,4-dinitrophenylhydrazine in phosphoric acid (300 ml), and the mixture was warmed in a boiling water-bath for 7 min. Orange–red ppt was filtered, recrystallized from dioxane, and identified with **6**.

D-*Glucose* 2,4-*dinitrophenylosazone tetraacetate* **7**. Ac_2O (0·6 ml) and pyridine (0·5 ml) was added to **6** (50 mg), and the resultant soln was left stand overnight, and then treated with ice-water. Ppt was filtered, dissolved in $CHCl_3$, and chromatographed on a silicic acid column (1 × 13 cm). The fraction which formed an orange band on the column was evaporated, and the residue was recrystallized from CH_2Cl_2-benzene, m.p. 136·5–139·5° (dec). $\lambda_{max}^{CHCl_3}$ (log ϵ) 392 (4·62) and 440 mμ (4·66). NMR (60 MHz, $CDCl_3$) δ 1·86, 2·02, 2·22, 2·31 (Ac), 4·0 ~ 4·7 (m, C_6-H × 2), 5·2 ~ 5·6 (m, C_5-H), 5·69 and 5·82 (d.d, $J = 2$

Hz, C_4-H), 6·72 (d, J = 2 Hz, C_3-H), 7·94 (s, C_1-H), 8·02 and 8·41 (ABq, J = 10 Hz, C_6-H and C_5-H), 9·12 (d, J = 2 Hz, C_3-H), 11·48 (NH), 12·67 (NH). Found: C, 44·19; H, 3·88; N, 15·59. $C_{26}H_{26}O_{16}N_8$ requires: C, 44·19; H, 3·71; N, 15·86%.

Periodic acid oxidation of D-*glucose 2,4-dinitrophenylosazone* **6**. A soln of **6** (300 mg) in dioxane (60 ml) was treated with $HIO_4.2H_2O$ (98·3%, 445 mg) in water (10 ml) for 24 h. The excess periodic acid was determined in 0·5 ml portion of the supernatant soln in the same manner as the periodic acid oxidation of **2**. The consumption per mole of **6** corresponded to 3·03 mole. The soln was concentrated to a half volume *in vacuo*, and water was added. The orange–red ppt was filtered, dried *in vacuo* (166 mg) and chromatographed on a silicic acid column (50 g) eluting with CH_2Cl_2, and then recrystallized from dioxane (52·3 mg), m.p. 260–264° (dec). $\lambda_{max}^{CHCl_3}$ (log ε) 340 (4·29) and 460 mμ (4·31). Found: C, 40·70; H, 2·36, N, 25·06. $C_{15}H_{10}O_9N_8$ requires: C, 40·37; H, 2·26; N, 25·11%.

Reaction of coriose with phenylhydrazine. Coriose (100 mg) was dissolved in a soln of phenylhydrazine (0·3 ml) in a mixture of AcOH (0·2 ml) and water (1·0 ml), and the soln was warmed in a boiling water-bath for 3 h. Dark red oil appeared in the soln, and solidified on cooling. This solid was washed with a few drops of MeOH, and identified as β-acetylphenylhydrazine by TLC and IR. The tar removed from acetyl phenylhydrazine showed on TLC more than ten spots, and gave no crystalline product by the chromatography on a silicic acid column. The reaction of coriose with phenylhydrazine in a mixture of AcOH and methylcellosolve also did not give a crystalline osazone.

Production of 1-*deoxycoriosone* **10** *from coriose* **1**. (a) A soln of coriose (50 mg) in 2N HCl (3·5 ml) was warmed in boiling water-bath for 1 h, and then cooled down to the room temp. A soln of 2,4-dinitrophenylhydrazine (165 mg) in warm 2N HCl (20 ml) was cooled to the room temperature, and added to the soln of acid-treated coriose. Orange–yellow ppt started to appear immediately, filtered and dried *in vacuo* (28·4 mg). This ppt was identified as **2**.

(b) A soln of coriose (50 mg) in 2N HCl (10 ml) was warmed in a boiling water-bath for 1 h, and after cooling, filtered soln was passed through a column of Amberlite IR-45 (10 ml). The eluant was concentrated *in vacuo* at 40°. The residual syrup showed on PC (ascending method, n-BuOH–pyridine–H_2O, 6:4:3) an orange spot (R_f 0·64) when sprayed with 2,4-dinitrophenylhydrazine reagent. Two spots were observed with sodium metaperiodate-benzidine at R_f 0·64 and 0·48. The latter spot was identified with coriose on PPC with orcinol reagent (orcinol–Cl_3CCOOH–n-BuOH, 1:30:240, w/w/v). The product corresponding R_f 0·64 was purified by preparative PC (syrup, 51 mg), and further purification by the second preparative PC yielded a syrup which showed a single spot on PPC, and a main peak on GLC at R_{Glu} = 0·39 (column temp. 160°). The purified syrup yielded **2** on addition of 2,4-dinitrophenylhydrazine reagent.

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