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Synthesis, structural properties and insulin-enhancing potential of bis(quercetinato)oxovanadium(IV) conjugate

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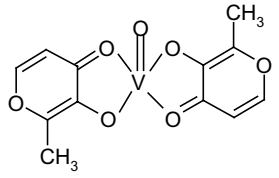
Abstract—An oxovanadium complex of quercetin (**2**), exhibits highly potent insulin-enhancing activity in streptozotocin-induced diabetic mice. It also mimics mitogenic potential of insulin as evaluated by [H^3]thymidine uptake assay making an effective, orally active insulin-enhancing agent for the treatment of both type 1 and type 2 diabetes without any noticeable toxic effects.

1. Introduction

The insulin-enhancing activity of vanadium compounds is well-established in laboratory animals as well as in humans in recent years.[1] Their recommended use in both type 1 (insulin-dependent) as well as in type 2 (non-insulin-dependent) diabetes is due to their ability to lower blood glucose levels by normalizing body carbohydrate as well as lipid metabolism.[2–5] Additionally they have been found to increase tissue insulin sensitivity.[6,7] Compounds containing three different physiologically relevant oxidation states of vanadium, viz., V^{+5}, V^{+4} and V^{+3}, are currently being explored as orally active insulin-enhancing therapeutics for diabetes, wherein the tetravalent vanadium compounds have especially been found more effective as blood glucose-lowering agents than other analogues.[8] Among these, bis(maltolato)oxovanadium(IV) (BMOV, **1**) is the most successful insulin-mimetic agent, which has been approved for clinical use.[9]



bis(maltolato)oxovanadium(IV) (BMOV, **1**)

However, pharmacologically beneficial dose of BMOV is associated with some toxicity and hence attempts are underway to evolve vanadium conjugates with less adverse effects while retaining their enhanced therapeutic activities.[10] Vanadium compounds examined for such purpose include ligands like pyridone,[8] picolinic acid,[11] sulfur ligands like cysteinale methyl ester,[12] pyrrolidine-N-carbodithionate[13] and thiazolidinediones,[14] acetylacetonates,[15] β-hydroxymates,[16] biguanides[17] and complexes of known hypoglycemic agents such as metformin.[18] Some of these are orally active in animal models of diabetes. This motivated us to search for more effective and efficient anti-diabetic ligands amongst naturally occurring, low molecular weight, polyphenolic compounds, which contain pyrone as the basic structural motif similar to that of maltol. Amongst these the most promising compound turned out to be quercetin (**2**), which is a common constituent of many vegetables and fruits including an Indian medicinal plant, *Rosa centifolia*.[19] In experimental animal model **2** has been found to alleviate not only the primary symptoms of diabetes but also the secondary complications[20] as well as inhibitory activities against viral infections, inflammatory conditions and malignant growths.[19] Treatment with **2** has been shown to reverse the deleterious effects of diabetes on the oxidized glutathione levels in brain and on the glutathione peroxidase activity in liver.[21] There have been speculations that the anti-diabetic activity of **2** may involve pancreatic β-cell regeneration.[22]

Keywords: Quercetin; Vanadium complex; Insulin mimic; Diabetes.
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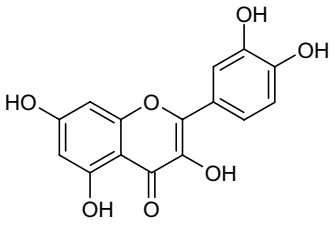
In the present communication we describe synthesis and structural characterization of vanadium complex of **2** that exhibits enhanced anti-diabetic properties than the parent ligand in streptozotocin-induced diabetes in mice without noticeable toxicity.

2. Experimental

Quercetin was isolated from *R. centifolia* by literature protocol.[23] Vanadyl sulfate ($VOSO_4{\cdot}5H_2O$) was obtained from Thomas Baker while maltol was obtained form Sigma Chemicals and these were used without further purification. Analytical grade solvents were employed during all syntheses, which were distilled prior to their use.



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The molecular weight of the complex was determined by direct probe mass spectrum on Shimadzu GCMS-QP 5050A machine. IR spectra were recorded in KBr discs in the range 4000–500 cm^{-1} on FTIR-8400 infrared spectrophotometer. Electronic spectra in the range 300–1100 nm were recorded on UV-1601 UV–vis spectrophotometer. Conductivity of the metal complex was measured on a conductivity instrument EQ-664 (Equiptronics) and was calibrated by using 0.1 M KCl in appropriate solvents prior to use. The EPR spectrum was recorded on a Varian E109 spectrometer at 77 K using TCNE compound as a calibrant. The magnetic susceptibility of the vanadium complex was measured at 300 K on a Faraday balance having field strength of 7000 G. Cyclic voltametric measurements were made in dimethylsulfoxide (DMSO) solvent on a Bioanalytical System BAS CV-27 instrument with an XY recorder using Pt disc as the working electrode against SCE and Pt wire as an auxiliary electrode. Tetraethyl ammonium perchlorate (TEAP) was used as a supporting electrolyte.

3. Synthesis

BMOV was prepared according to the method described earlier.[24] The vanadium complex of **2** was synthesized by refluxing a mixture of methanolic solutions of **2** and $VOSO_4{\cdot}5H_2O$ on a water bath for 3 h and stripping off the solvent on a rotary evaporator when the brown vanadium complex separated out. The compound was filtered out, washed with cold methanol and dried in vacuum desiccator. Yield = 60%.

4. Cell culture and biological assays

The glucose-lowering properties of the compound were examined in streptozotocin-induced diabetes model. Male Balb/c mice 6–8 weeks old were made diabetic by intraperitoneal injection of streptozotocin (180 mg kg^{-1} bodyweight) freshly dissolved in chilled sodium citrate buffer (pH 4.5). Mice showing blood glucose above 200 mg dL^{-1} were taken for further experiments as diabetic mice. The test compounds and BMOV (as standard vanadium compound) were administered orally at a dose of 0.4 mmol kg^{-1} bodyweight[25] and compared for their glucose-lowering potential. An appropriate vehicle control (DMSO) was also run. Blood samples were collected from tail vein just prior to drug administration and then at regular intervals until 24 h for blood glucose analysis by Accu-chek blood glucose analyzer (Roche). Effect of the vanadium complex of quercetin was also determined on normal Balb/c mice following an identical procedure.

In addition to the hypoglycemic activity the mitogenic potential of the test compounds was also examined.[26] Finally capabilities of all test compounds and the parent ligand moiety in inducing proliferation in serum starved CHO cells were evaluated at various concentrations by [H^3]thymidine uptake assay. Typically CHO cells were seeded in 96-well plate at a cell density of 2×10^4 cells/well and allowed to attach for 6 h in presence of MEM(E) supplemented with 10% FCS. Cells were then serum starved for 16 h to allow the cells to become quiescent. Test compounds were then added to the cells at indicated concentrations and were allowed to incubate for 24 h followed by addition of [H^3]thymidine (1 μCi mL^{-1}) to all the wells for next 6 h at 37 °C. The cells were then detached using trypsin–EDTA solution and harvested by Filtermate harvester, Packard, Bioscience. The incorporation of [H^3]thymidine into DNA was determined by using microplate scintillation and luminescence counter, Topcount. NXT (Packard, Bioscience).

5. Compositional studies

The metal to ligand stoichiometry of the vanadium conjugate of **2** is found to be 1:1 with a molecular weight of 868 Da. The compound is electrically neutral as revealed from the conductivity measurements in DMSO solvent.

6. Spectroscopy and magnetism

The infrared spectra of the parent ligand quercetin, is found to exhibit the characteristic carbonyl stretching frequency around 1665 cm^{-1}, which is shifted to 1634 cm^{-1} upon metal complexation indicating involvement of the carbonyl oxygen in metal coordination similar to that observed in case of transition metal complexes of analogous flavonoid ligand.[27] The characteristic ν(V=O) stretch in the oxovanadium compounds is generally observed in the range 950–995 cm^{-1}.[28] In the present compound it is observed at 990 cm^{-1}.

The electronic spectrum of the ligand recorded in MeOH exhibits two bands at 378 nm (Band I) related to B ring (cinnamoyl system) and 261 nm (Band II) related to ring A (benzoyl system) of the flavonoid moiety, respectively. The presence of *o*-dihydroxyl group in the B ring is suggested by the bathochromic shift of about 55 nm observed for Band I, which also indicates that they are not involved in metal complexation. This lends support to the participation of 3-hydroxyl group in metal coordination. The ligand-to-metal charge transfer (LMCT) band in the present vanadium compound can be seen at 430 nm.

The spin only value of the magnetic moment of oxovanadium complexes are found to be close to the value of 1.73 BM, although there are many donor atom systems like Schiff base complexes derived from salicylaldehyde that exhibit subnormal magnetic moments.[29] The room temperature magnetic moment of 1.55 BM observed for the present compound suggests a dimeric association, which is supported by its solution EPR spectrum showing 13 metal hyperfine lines with $g_{\|}$ and $g_{\perp}$ values of 1.99 and 2.05, respectively.

7. Cyclic voltammetry

The cyclic voltametric profiles of **2** and its vanadium complex in DMSO solvent are shown in Figure 1. The two oxidation peaks for the flavonoid ligand at +0.81 and +1.1 V correspond to the conversion of catecholic hydroxyls in the B ring to their quinonoidal form and the intramolecular rearrangement resulting in the deprotonation of the C-3 hydroxyl group as reported by Bodini et al.[30]

Upon metal complexation these oxidation peaks are found to disappear indicating perhaps stabilization of the catecholic hydroxyls and involvement of the deprotonated C-3-hydroxyl group in metal coordination. The additional quasi-reversible wave observed at $E_{1/2} = -0.48$ V is ascribed to V^{IV}/V^{V} redox couple.[25]

8. Hypoglycemic and mitogenic potential

Although blood glucose levels are generally down-regulated upon administration of anti-diabetic drugs, the percentage decrease and time course of such lowering varies depending upon nature and characteristics of the drug. In our study the vehicle control (DMSO) did not show any significant effect on blood glucose levels in diabetic mice while the vanadium complex showed maximum reduction at the end of 21 h after which the levels started rising slowly again. Maximum reduction of blood glucose was observed for BMOV, $VOSO_4$ and the parent ligand **2** at the end of 24 h period. Consequently 21 h time-point was taken as the optimum time for comparison of the glucose-lowering ability of all compounds (Fig. 2).

The highest reduction in blood glucose level (60%) after 21 h is found in case of the present vanadium compound while 30% reduction is observed for BMOV.[25] The percentage reduction in case of vanadyl sulfate and **2** are 14% and 20%, respectively. It is interesting to note that the present vanadium conjugate had no effect on blood glucose level of normal mice (data not shown). It should also be noted that not all diabetic mice respond uniformly to the vanadium–quercetin conjugate. For example, in some cases a pronounced reduction in plasma glucose (40–60%) is observed until the point of euglycemia while in others there is not much significant reduction and hence the experimental mice population can be divided into two groups of animals, viz. responders and nonresponders as observed in case of BMOV treatment.[3] The results shown in Figure 2 thus represent the responder population.

Since superior activity of the present vanadium compound would allow for the lowered dose regimen in therapeutic formulations, it may thus offer benefits in terms of long term toxicity. The higher antioxidant potential of **2** is an additional advantage in case of the present vanadium compound.[21]

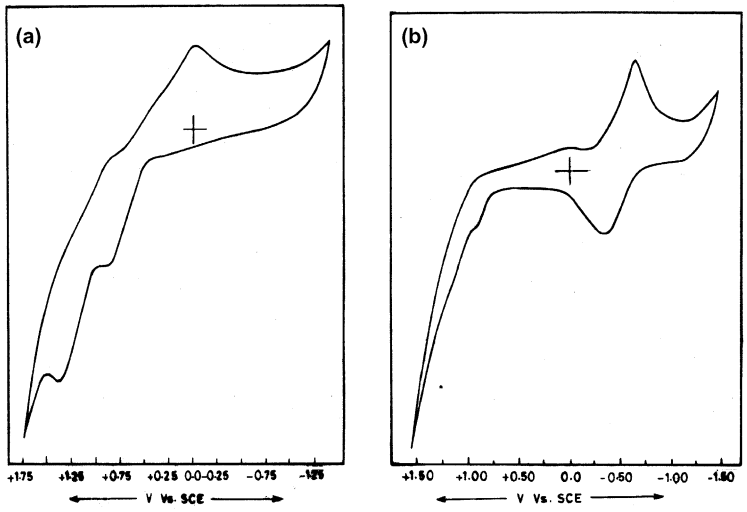

Figure 1. Cyclic voltammograms of **2** and its vanadium conjugate in DMSO.

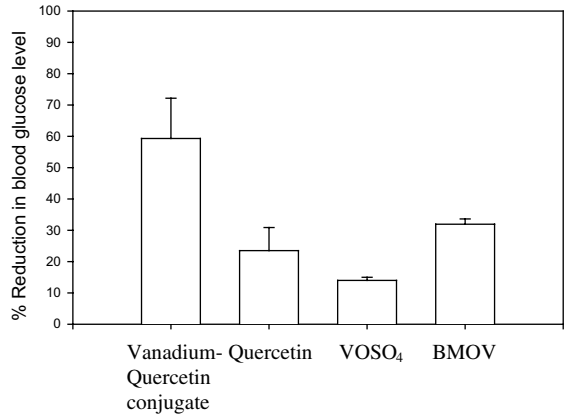

Figure 2. Hypoglycemic activity of **2** and its vanadium conjugate compared with BMOV and $VOSO_4$. Compounds were administered orally at a one-time dose of 0.4 mmol kg^{-1} to diabetic mice. Blood glucose was measured just before and at 21 h after the dosing. % Reduction of blood glucose = (initial level – final level)/initial level × 100. Values represent mean ±SD, $n = 8$. Mice treated with vanadium–quercetin conjugate and BMOV were compared by *t*-test ($p = 0.006$). Data represents only the responder population.

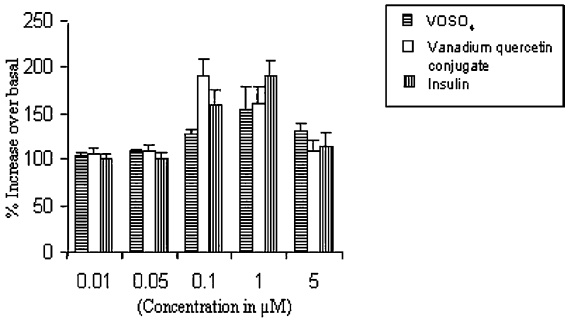

Figure 3. Cell proliferative activities of $VOSO_4$, vanadium–quercetin conjugate and insulin. Serum starved CHO cells were treated with various concentrations of $VOSO_4$, vanadium–quercetin conjugate and insulin (from 0.01 to 5 μM) for 24 h and were evaluated for cell proliferation by [H^3]thymidine incorporation. Data represents % increase over basal (mean ± SE), taking basal level as 100%.

In further experiments it was observed that cellular proliferation is not affected by **2** (0.01–5 μM) or DMSO vehicle controls although, at higher concentrations **2** is known to inhibit cellular proliferation.[31] The vanadium–quercetin complex as well as $VOSO_4$ are able to induce proliferation in CHO cells (Fig. 3) although the former has a much higher potential than $VOSO_4$, which is comparable with that of insulin at 0.1 μM concentration.

In conclusion, the present study reveals that the vanadium complex of a naturally occurring flavonoid, **2** is a potential oral insulin mimetic agent of therapeutic value due to its hypoglycemic and mitogenic activity in both type 1 and type 2 diabetes.

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References and notes

1. Shechter, Y. *Diabetes* **1990**, *39*, 1.
2. Valera, A.; Rodriguez-Gil, J. E.; Bosch, F. *J. Clin. Invest.* **1993**, *92*, 4.
3. Marzban, L.; Rahimian, R.; Brownsey, R. W.; McNeill, J. H. *Endocrinology* **2002**, *143*, 4636.
4. Schechter, Y.; Shisheva, A. *Endeavour, New Sci.* **1993**, *17*, 27.
5. Thompson, K. H.; Yuen, V. G.; McNeill, J.; Orvig, C. In *Vanadium Compounds; Chemistry, Biochemistry and Therapeutic Applications*; Tracey, A. S., Crans, D. C., Eds.; Oxford University Press: New York, 1998; pp 329–343.
6. Cohen, N.; Halberstam, M.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. J. *Clin. Invest.* **1995**, *95*, 2501.
7. Marita, A. R.; Anilkumar, K. L. *Diabetes Obes. Metab.* **2001**, *3*, 3271.
8. Thompson, K. H.; Orvig, C. *J. Chem. Soc., Dalton Trans.* **2000**, 2885.
9. Yuen, V. G.; Orvig, C.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1995**, *73*, 55.
10. Thompson, K. H.; Orvig, C. *Science* **2003**, *30*, 936.
11. Melchiar, M.; Thompson, K. H.; Jong, J. M.; Rettig, S. J.; Schutter, E.; Yuen, V. G.; Zhou, Y.; McNeill, J. H.; Orvig, C. *Inorg. Chem.* **1999**, *38*, 2288.
12. Sakurai, H.; Hamada, Y.; Shimomura, S.; Yamashita, S.; Ishizu, K. *Inorg. Chim. Acta* **1980**, *46*, 1–19.
13. McCormick, B. *J. Inorg. Chem.* **1968**, *7*, 1965.
14. Storr, T.; Mitchell, D.; Buglyo, P.; Thompson, K. H.; Yuen, V. G.; McNeill, J. H.; Orvig, C. *Bioconjugate Chem.* **2003**, *14*, 212.
15. Durai, N.; Swaminathan, G. *J. Chin. Biochem. Nutr.* **1997**, *22*, 31.
16. Goldwaser, I.; Li, J.; Gershonov, E.; Armoni, M.; Karnieli, E.; Fridkin, M.; Shechter, Y. *J. Biol. Chem.* **1999**, *274*, 26617.
17. Woo, L. C. Y.; Yuen, G.; Thompson, K. H.; McNeill, J. H.; Orvig, C. *J. Inorg. Biochem.* **1999**, *76*, 251.
18. Kandaswami, C.; Middleton, E., Jr. *Adv. Exp. Med. Biol.* **1994**, *366*, 351.
19. Harborne, J. B. *The Flavonoids: Advances in Research Since 1980*; Chapman and Hall: London, 1988.
20. Varma, S. D.; Mizuno, A.; Kinoshita, J. H. *Science* **1977**, *195*, 205.
21. Sanders, R. A.; Rauscher, F. M.; Watkins, J. B., III *J. Biochem. Mol. Toxicol.* **2001**, *15*, 143.
22. (a) Vessal, M.; Hemmati, M.; Vasei, M. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* **2003**, *135*, 357; (b) Pederson, R. A.; Ramanadhan, S.; Buchan, A. M. J.; McNeill, J. H. *Diabetes* **1989**, *38*, 1390.
23. Geissman, T. A. *The Chemistry of Flavonoid Compounds*; Pergamon: London, 1962.
24. McNeill, J. H.; Yuen, V. G.; Hoveyda, H. R.; Orvig, C. *J. Med. Chem.* **1992**, *35*, 1489.
25. Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H.; McNeill, J. H.; Pettig, S. J.; Setyawati, I. S.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759.
26. Barrio, D. A.; Williams, P. A.; Cortizo, A. M.; Etcheverry, S. B. *J. Biol. Inorg. Chem.* **2003**, *8*, 459.

27. Zhou, J.; Wang, L.; Wang, J.; Tang, N. *Trans. Met. Chem.* **2001**, *26*, 57.
28. (a) Plass, W. *Angew. Chem., Int. Ed.* **1996**, *35*, 627; (b) Plass, W. *Z. Anorg. Allg. Chem.* **1997**, *623*, 1290; (c) Plass, W. *Eur. J. Inorg. Chem.* **1998**, 799.
29. Ginsberg, A. P. *Inorg. Chem.* **1966**, *5*, 1656.
30. Bodini, M. E.; Copia, G.; Tapia, R.; Leighton, F.; Herrera, L. *Polyhedron* **1999**, *18*, 2233.
31. Gliszczynska-Swiglo, A.; van der Woude, H.; de Haan, L.; Tyrakowska, B.; Aarts, J. M.; Rietjens, I. M. *Toxicol. In Vitro* **2003**, *17*, 423.